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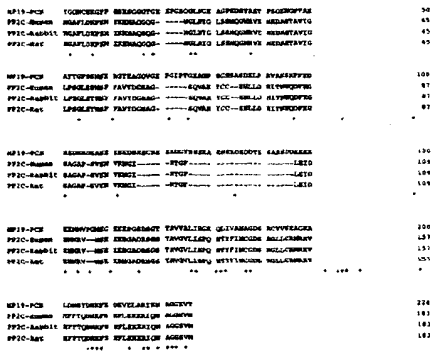
Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Nucleic acid encoding a human protein phosphatase

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Fig. 1



EP 0 874 052 A2

Description

The present invention relates to nucleic acids encoding a novel human protein phosphatase of the protein serine/threonine phosphatase family. In particular, it relates to novel DNA sequences encoding a serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Protein phosphorylation-dephosphorylation is a universal mechanism by which different cellular events are regulated. The serine/threonine-specific phosphatases have been classified into four main types according to their in vitro specificity for selected substrates and sensitivity to activators and inhibitors (Ingebritsen, T.S. and Cohen, P. (1983) Eur. J. Biochem. 182, 255-261). Sequence analysis revealed that they can be classified into two major gene families. The first one includes type 1 (PP1), type 2A (PP2A), and type 2B (PP2B) phosphatases, which share 37 to 59 % sequence identity (Barton, G.J. et al., (1994) Eur. J. Biochem. 220, 225-237) in their catalytic domains and are inhibited by okadaic acid (Bialojan, C., and Takai, A. (1988) Biochem. J. 256, 283-290). The second family, the Mg^{2+}-dependent phosphatases, also designated type 2C (PP2C), share little sequence similarity with the first family and are insensitive to okadaic acid. cDNA sequences of PP2C α and β from mammalian sources showed > 90 % identity. PP2Cs have been implicated in the regulation of fatty acid and cholesterol biosynthesis (Moore, F. et al. (1991) Eur. J. Biochem. 199, 691-697) and heat shock response (Maeda et al. (1993) Mol. Cell. Biol. 113, 5408-5417, Shiozaki, K. et al. (1994) Mol. Cell. Biol. 14, 3742-375).

The technical problem underlying the present invention is to provide a new human PP2C-like protein phosphatase which is distinct from the other PP2Cs.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims. Other features and advantages of the invention will be apparent from the description of the preferred embodiments and drawings.

The sequence listings and drawings will now be briefly described.

SEQ ID NO. 1 shows the nucleotide sequence of MP-19, a DNA sequence derived from human placenta.

SEQ ID NO. 2 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 1.

SEQ ID NO. 3 shows the nucleotid sequence of MP-19 full-lenght cDNA derived from human placenta.

SEQ ID NO. 4 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 3.

The figures show:

Figure 1 shows the alignment of the amino acid sequence of MP-19 with some related proteins of the PP2C family. The asterisk (*) indicates the position of identical amino acids of the compared amino acid sequences.

MP19-PCR:	amino acid sequence of MP-19 (SEQ ID NO. 2)
PP2C-Human:	human protein phosphatase 2C alpha (Accession No. S87759)
PP2C-Rabbit:	rabbit protein phosphatase 2C alpha (Accession No. S87757)
PP2C-Rat:	rat protein phosphatase 2C (Accession No. J04503)

Figure 2 is a human RNA master blot. The RNA-dot blot analysis shows hybridization of MP-19 PCR probe with different human RNA samples.
A1 whole brain, A2 amygdala, A3 caudate nucleus, A4 cerebellum, A5 cerebral cortex, A6 frontal lobe, A7 hippocampus, A8 medulla oblongata, B1 occipital lobe, B2 putamen, B3 substantia nigra, B4 temporal lobe, B5 thalamus, B6 subthalamic nucleus, B7 spinal cord, B8 blank, C1 heart, C2 aorta, C3 skeletal muscle, C4 colon, C5 bladder, C6 uterus, C7 prostate, C8 stomach, D1 tests, D2 ovary, D3 pancreas, D4 pituitary gland, D5 adrenal gland, D6 thyroid gland, D7 salivary gland, D8 mammary gland, E1 kidney, E2 liver, E3 small intestine, E4 spleen, E5 thymus, E6 peripheral leukocyte, E7 lymph node, E8 bone marrow, F1 appendix, F2 lung, F3 trachea, F4 placenta, F5-F8 blank, G1 fetal brain, G2 fetal heart, G3 fetal kidney, G4 fetal liver, G5 fetal spleen, G6 fetal thymus, G7 fetal lung, G8 blank, H1 yeast total RNA, H2 yeast tRNA, H3 *E. coli* rRNA, H4 *E. coli* DNA, H5 poly r(A), H6-H8 human DNA.

Figure 3 shows the detection of MP-19 after IMAC. The Western analysis was performed after purification of MP-19 using Immobilized Metal Ion Affinity Chromatography (IMAC). Positive signals were obtained from fraction 16 to fraction 22.

The amino acid sequence alignment of MP-19 with sequences of different PP2Cs (shown in Figure 1) demonstrates the homology of MP-19 to the PP2C family but implicate also that MP-19 belongs to a new protein phosphatase group. The homology of the derived MP-19 amino acid sequence (aa 1 - aa 226) to PP2C from human, rabbit and rat displays a sequence homology of 21.2 %. Moreover a partial sequence of MP-19 (amino acid sequence 158 - 226) which is in the shown alignment not disrupted by gaps indicates a sequence homology of 39.1 % to the compared PP2Cs.

The present invention relates particularly to a novel serine/threonine protein phosphatase and, preferably, provides DNA sequences contained in the corresponding gene.

Such sequences include nucleotide sequences as illustrated in SEQ ID NO. 1 and SEQ ID NO. 3, allelic derivatives of said sequences and DNA sequences degenerated as a result of the genetic code for said sequences. It also includes DNA sequences hybridizing under stringent conditions with the DNA sequence mentioned above. It further includes antisense nucleic acid, preferably antisense MP-19 nucleic acid, directed to the above defined nucleic acid. The terms "nucleic acid sequence" and "nucleotide sequence" refers to DNA or RNA or heterooligomeric sequences, which may be double- or single-stranded.

Although said allelic, degenerate and hybridizing sequences may have structural divergences due to naturally occurring mutations, such as small deletions or substitutions, they will usually still exhibit essentially the same useful properties, allowing their use in basically the same medical or diagnostic applications.

According to the present invention, the term "hybridization" means conventional hybridization conditions, preferably conditions with a salt concentration of 6 x SSC at 62°C to 66°C followed by a one-hour wash with 0.6 x SSC, 0.1% SDS at 62°C to 66°C.

Important biological embodiments of the present invention are DNA sequences of the above and obtainable from vertebrates, preferably mammals such as pig and from rodents such as rat, and in particular from primates such as humans.

Particularly preferred embodiments of the present invention are the DNA sequence termed MP-19 which are shown in SEQ ID NO. 1 and SEQ ID No. 3. The corresponding transcripts of MP-19 were obtained from human placenta tissue and code for a protein showing considerable amino acid homology to the PP2C proteins (shown in Figure 1 deduced from SEQ ID NO. 1). The protein sequence of rabbit and human PP2C α and rat and rabbit PP2C α are described in Mann et al. (1992) Biochim. Biophys. Acta 1130, 100-104. Some typical sequence homologies, which are specific for the known PP2Cs, were also found in the MP-19 sequence. In the present invention, cloning was carried out according to the method described below. Once the DNA sequence has been cloned, the preparation of host cells capable of producing the PP2C-like protein MP-19 and the production of said protein can be easily accomplished using known recombinant DNA techniques comprising constructing the expression plasmids encoding said protein and transforming a host cell with said expression plasmid, cultivating the transformant in a suitable culture medium, and recovering the product having PP2C-like activity.

Thus, the invention also relates to recombinant molecules comprising DNA sequences as described above, optionally linked to an expression control sequence. Such vectors may be useful in the production of the PP2C-like protein in stable or transiently transformed cells. Several animal, plant, fungal and bacterial systems may be employed for the transformation and subsequent cultivation process. Preferably, expression vectors which can be used in the invention contain sequences necessary for the replication in the host cell and are autonomously replicable. It is also preferable to use vectors containing selectable marker genes which can be easily selected for transformed cells. The necessary operation is well-known to those skilled in the art.

It is another object of the invention to provide a host cell transformed by an expression plasmid of the invention and capable of producing a protein of the serine/threonine phosphatase family. Examples of suitable host cells include various eucaryotic and procaryotic cells, such as *E. coli*, insect cells, plant cells, mammalian cells, and fungi such as yeast.

Another object of the present invention is to provide a PP2C-like protein or a biologically active fragment thereof encoded by the sequences described above and displaying biological features such as dependency of Mg^{2+} (or Mn^{2+}) for activity. Furthermore, the phosphatase catalyzes dephosphorylation of phosphoseryl/threonyl residues of proteins and peptides phosphorylated by cAMP-dependent protein kinases and protein kinase C. It is insensitive to inhibitors like okadaic acid and calyculin A, heparin and PP1 inhibitors 1 and 2. It does not attack phosphorylase α. It is inhibited by polycations and F^- ions. A preferred substrate for the PP2C-like protein is the SET protein, suggesting capacities possible relevant to therapeutically treatment of leukemia. Furthermore, the PP2C-like protein prefers basic substrates such as histones, and MBP phosphorylated by cAMP-dependent protein kinase, suggesting a special function for this phosphatase in the brain. The amino acid sequences of especially preferred PP2C-like proteins (MP-19) are shown in SEQ ID NO. 2 and SEQ ID NO. 4.

It is a further aspect of the invention to provide a process for the production of PP2C-like proteins. Such a process comprises cultivating a host cell being transformed with a nucleic acid sequence of the present invention in a suitable culture medium and purifying the PP2C-like protein produced. Thus, this process allows the production of the sufficient amount of the desired protein for use in medical treatments. The host cell is obtainable from bacteria such as *Bacillus spec.* or *Escherichia coli*, from fungi such as yeast, from plants such as tobacco, potato, or *Arabidopsis*, and from animals, in particular vertebrate cell lines such as the Mo-, COS- or CHO cell line.

It is another object of the present invention to provide pharmaceutical compositions containing a therapeutically-effective amount of a PP2C-like protein of the present invention and, optionally, a pharmaceutically acceptable carrier and/or diluent, and/or agonists and/or antagonists thereof. Such a therapeutic composition can be used for the treatment of cancer such as leukemia, brain cancer, breast cancer and prostate cancer. The pharmaceutical composition according to the present invention can also be therapeutically applied for degenerative disorders of the CNS, e.g. Alzheimer's disease, Huntingdon's disease, Parkinson's disease, and epilepsy, and disorders of the reproductive system e.g. fertility disorders or testicular cancer. Another possible clinical application of a PP2C-like protein is the use for treatment of liver diseases, diabetes, and cystic fibrosis. The pharmaceutical composition comprising the protein of the present invention can also be used for treatment of microbial or viral infections. Another application of the pharmaceutical composition of the present invention is the usage of the protein in the regulation of spermatogenesis or the maturation of mammalian germ cells, e.g. for centraception.

Furthermore, the application of the composition is not limited to humans but can include animals, in particular domestic animals, as well.

Finally, another object of the present invention is an antibody or antibody fragment, which is capable of specifically binding to the proteins of the present invention. Methods to raise such specific antibody are known in the art. Such an antibody is preferably a monoclonal antibody. Such antibodies or antibody fragments might be useful for diagnostic methods.

The following examples illustrate in more detail the present invention, but should not be construed as limiting the invention.

Example 1

Isolation of MP-19

For the reverse transcription reaction, 5 µg total RNA (0.5 µg/µl) derived from human placenta tissue was heated for 5 minutes and cooled rapidly on ice for 5 minutes. The reverse transcription reagent mixture containing 5 µg total RNA, 38 u of RNA-guard (Pharmacia), 2.5 µg oligomer d(T)12-18 (Boehringer Mannheim), 5x reaction buffer (250 mM Tris/HCl pH 8.5; 50 mM $MgCl_2$; 50 mM DTT; 600 mM KCl), 10 mM of each dNTP (Pharmacia), 37.5 u of avian myoblastosis virus reverse transcriptase (AMV, Boehringer Mannheim). The reaction mixture (20 µl) was incubated for 90 minutes at 42°C. The resulting placenta cDNA pool was stored at -20°C.

For the primary polymerase chain reaction (PCR), a placenta-derived cDNA pool was used as template in a 50 µl reaction mixture. The PCR reaction was carried out in a RoboCycler Gradient 96 (Stratagene). The amplification was performed in 1x PCR-buffer (10 mM Tris/HCl pH 8.3; 50 mM KCl; 0.001 % gelatine), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide (ALK6-N2, 5' - TT(CT)(AG)C(AGCT)AT(AGCT)ATAGAAGAAGATGA - 3' and ALK6-R2, 5' - CC(AGCT)CGCCA(CT)TT(AGCT)CCCATCCA - 3') and 1.5 u Taq polymerase (Perkin Elmer). The PCR reaction contained cDNA corresponding to 30 ng of total RNA as starting material. The reaction mixture was overlaid by 40 µl paraffin incubated for 180s/94°C and subjected to 30 cycles (50s/94°C, 90s/48 °C, 60s/72 °C) with an additional extension for 480s/72 °C in the Thermocycler.

A second round of amplification was performed as described above with exception that 5 µl from the first PCR reaction was used as template DNA for the PCR. A 10 µl sample from the second PCR amplification was fractionated by electrophoresis using a 2 % agarose gel in TBE buffer. After electrophoresis amplified DNA corresponding to a molecular weight of about 600-800 bp was excised from the gel and isolated by 3x freeze/thaw cycles (-20 °C/ + 37 °C) and using the DNA Purification Kit "Easy Pure" (Biozyme, Cat. no. 39001) following the instructions of the manufacturer.

The eluted DNA was amplified a third time as described in the primary PCR with exception that 3 µl of the eluted DNA, resulted from the second round of amplification, was used as template for PCR and the annealing temperature was 56 °C instead of 48 °C. After electrophoresis using a 2% agarose gel in TBE buffer, a distinct DNA band that corresponds to a molecular weight of about 700 bp was eluted with the extraction method described before. After than an additionally purification using the QIAquick 8 PCR Purification Kit (Qiagen, Cat. no. 28144) following the instructions of the manufacturer, was carried out.

Cloning of the purified DNA was established using the Original TA Cloning Kit (Invitrogen, Cat. no. K2000-40). Plasmid DNA from positive clones was isolated with the QIAwell 8 Plus Plasmid Kit (Qiagen, Cat. no. 16142) and sequenced with an automatic DNA sequencer (ALFexpress, Pharmacia). The resulting DNA sequence was analyzed by a homol-

ogy search with the blast program.

Example 2

Isolation of MP-19 full-length cDNA

Isolation of a full-length cDNA clone of MP-19 was performed with a commercial available Human Placenta Lambda cDNA Library (Stratagene, Cat. no. 937225). For screening, a labeled PCR probe was generated from MP-19 DNA (SEQ ID NO. 1). The amplification was performed in 1x PCR-buffer (Qiagen, Germany), 1 mM of dATP, 1 mM of dCTP, 1 mM of dGTP, 0.6 mM of dTTP (Pharmacia, Germany), 0.4 mM of Digoxigenin-11-dUTP (Boehringer, Mannheim), 100 pmol of each oligonucleotide PL19-N1 (5'-GGGCAGAACTGTCACAAGGG-3') and PL19-R1 (5'-CATCCATGGTGACCTTGCCACC-3') and 1 u Taq DNA-polymerase (Qiagen). The PCR mix was overlaid by 40 µl paraffin, incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/58 °C, 60s/72 °C) with an additional extension for 180s/72 °C.

Prehybridization of plaque lift filters from cDNA library were done at 58 °C for 4 h in 0.25 M Na_2HPO_4, 7 % SDS, 1 % BSA, 1 mM EDTA, pH 7.2. Hybridization was carried out with 50 ng labeled MP19 PCR probe for 15 h under same buffer conditions as prehybridization was done. Filter washed 3 times (5 min, 10 min and 15 min) with 30 mM Na_2HPO_4, 0,1 % SDS at 60 °C. Detection of signals were performed with DIG Luminescent detection kit from Boehringer, Mannheim (Cat. no. 1363514). Proceed from a positive signal, clone 39-1 was isolated and sequenced. The resulting DNA-sequence from clone 39-1 conform to Bp. 387-1641 in SEQ ID NO. 3. To generate the 5'cDNA end of MP-19 full-lenght cDNA, 1 µg of a Lambda-DNA preparation from Human Placenta Lambda cDNA Library was subjected to PCR. The ampilfication was performed in 1x PCR-buffer (Qiagen), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide MP19-E5 (5'-GGATCCATGGGTGCCTACCTCTCCCAGCCC-3') which was derived from an EST sequence (accession no. AA115688) and MP19-3 (5'-GCCTGTGTAGGCCTTGGCTGTGGGGCC-3') and 1 u Taq DNA-polymerase (Qiagen). The reaction was overlaid by 40 µl paraffin incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/74 °C, 60s/72 °C) with an additional extension for 300s/72 °C. The resulting PCR fragment was subcloned in vector pCR 2.1 (Clontech, Germany). Corresponding DNA was digested with restriction endonucleases Bam HI and Stu I. After that MP-19 Bam HI/Stu I fragment was inserted into clone 39-1. The resulted clone was named 28-9 which DNA-sequence present the full-lenght cDNA-sequence of MP-19 as shown in SEQ ID NO. 3. The corresponding amino acid sequence of MP-19 is shown in SEQ ID NO. 4.

Gene expression of MP-19 in human tissues

Relative expression of the MP-19 gene was determined by Northern blot analyses. A commercial available Human RNA Master Blot (Clontech, Germany, Cat. no. 7770-1, Lot no. 7090716) was hybridized with the digoxigenin labeled MP-19 PCR probe as described in: Isolation of MP-19 full-length cDNA. 50 different human tissues samples were investigated for MP-19 gene expression. Additionally 8 different negative controls from *E. coli*, yeast and human genomic DNA were applied.

Main expression of MP-19 was detected in human testis which is shown in figure 2. Lower expression of MP-19 was detected in human pituitary gland, thymus, small intestine and fetal liver. Basal expression of MP-19 was found in all other human RNA samples. No hybridization signals were detected in negative controls.

Expression of MP-19 cDNA in *E. coli*

The cDNA of clone 28-9 was subcloned into the expression plasmid pQE-16 (Qiagen, Germany). This cloning strategy constituted an additional tag of 6 histidine residues at the C-terminus of MP-19. pQE-16 was digested with Bam HI and Bgl II. The 5' part of MP-19 was excised from clone 28-9 with Bam HI and Sac I. To constitute a compatible 3'end of MP-19 for cloning into plasmid pQE-16, a PCR was performed with primer MP19N-Sac I (5'-ACAGCAGAGCTCCAGCCAGAG-3') and MP19R-Bgl II (5'-AGATCTGTCTCGCTTGGCCTTCTTCTTC-3') and template DNA of clone 28-9.5' and 3'end of MP-19 DNA was ligated into pQE-16 to establish MP-19 with His-tag, which was expressed in *E. coli* strain M15 (Qiagen). For recombinant expression of MP-19, cells were grown in a 5 l fermenter (Bio Console ADI 1035, Applikon, Netherlands) at 37 °C in LB-Medium until an OD_{600} of 2,5 was reached. After induction with 1 mM β-D-thiogalactopyranoside, cells were grown for additional 4 h until OD_{600} of 9,7 was reached. Cells were harvested by centrifugation at 10.000 x g for 30 min, washed once in 500 ml 1 x PBS buffer (30 min at 10.000 x g) and were frozen in aliquots at -80 °C. For preparation of MP-19 protein, 10 g cells were lysed in 100 ml lysis buffer (50 mM NaH_2PO_4, pH 8,0, 300 mM NaCl, 10 mM imidazole, 100 mg lysozyme (Serva, Germany) and 50 u Benzonase (Merck, Germany)) following by sonication 3 times with a ultrasonic processor (UP-200S, Dr. Hielscher GmbH, Germany) for 3 min at 5 $kWsec^{-1}$ in an ice/water bath. Cell debris was removed by centrifugation for 30 min at 4 °C and 25.000 x g.

Purification of recombinant MP-19

Recombinant expressed MP-19 was purified using Immobilized Metal Ion Affinity Chromatography (IMAC) and Reverse Phase Liquid Chromatography (RPLC). The chromatographic purification was realized using the ÄKTA-Explorer system (Pharmacia Biotech, Germany). For IMAC, a 1 ml Hi-Trap cheating column (Pharmacia Biotech) is used. Hi-Trap column was activated with 5 column volumes (cv) of 100 mM $NiSO_4$, afterwards the column washed with 5 cv water to remove unbound Ni^{2+}. Column equilibration was performed with 5 cv of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazole, pH 8,0). Cell lysat results from 1 g *E. coli* cells was loaded onto column. Afterwards the column washed with lysis buffer to remove unbound protein. Protein were eluted using the following gradient program. Step 1: 20 mM imidazole to 300 mM imidzole within 20 minutes, step 2: 300 mM imidazol to 500 mM imidazol within 10 minutes using buffer A (50 mM Tris, 300 mM NaCl, 20 mM Imidzole, pH 6,0) and buffer B (50 mM Tris, 300 mM NaCl, 500 mM Imidazole, pH 6,0). Flow rate of chromatography were 1 ml/min, detection were performed at 280 nm. Fractions were analyzed by immunological detection, shown in Figure 3. For further purification, positive fractions containing MP-19 were pooled and loaded onto a Resource RPC (3ml) column (Pharmacia Biotech). Column was equilibrated with buffer A (0.1 % trifluor acetic acid) and protein eluted with a linear gradient of buffer B (0,1 % TFA-90 % acetonitrile). Flow rate of chromatography were 3 ml/min, detection were performed at 215 nm.

Immunolonical Detection of MP-19

Immunological detection of recombinant MP-19 was performed by western blotting using a commercial available monoclonal mouse antibody against histidine-tag (Dianova, Germany, Cat. no. dia900) in combination with Western Light chemoluminescent detection system using the goat anti-mouse-AP antibody (Tropix, U.S.A.).

Activity assay for recombinant MP-19

A Serine/Threonine Phosphatase Assay System (Promega, Germany, Cat. no. V2460) was used to determine enzymatic activity of recombinant MP-19. This assay use a chemically synthesized phosphopeptide RRA(pT)VA which is a functional substrate for MP-19 phosphatase. The amount of free phosphate which is generated by MP-19 enzymatic reaction was measured by the absorbency of a molybdate:malachit green:phosphate complex (Ekman P. and Jager O. (1993), Anal. Biochem. 214, 138-141, Deana A. D. et al. (1990), Biochimica et Biophysica Acta 1051, 199-202). Assays were performed as described by the manufacturer in PPTase-2C buffer (50 mM imidazole, pH 7,2, 0,2 mM EGTA, 5 mM $MgCl_2$, 0.02 % β-mercaptoethanol, 0,1 mg/ml BSA). To determine background of this assay, clone pQE-16-dhfr (Qiagen, Germany) was used, which is identical to pQE-MP-19 with exception that vector pQE-16 inserted a mouse dhfr gene instead of MP-19 phosphatase gene.

Results of activity assay of MP-19 shown in table 1. MP-19 has a significant activity in a $MgCl_2$ containing buffer, but no activity in a $CaCl_2$ containing buffer, which shows the requirement to Mg^{2+}. Inhibitors like okadaic acid (10 µM) shows no significant reduction of MP-19 activity. Control expression of the mouse dhfr gene shows no activity in the Serine/Threonine Phosphatase Assay System.

	A	B	C	D (Average A-C)
1	0.000	0.006	-0.006	0.000
2	0.024	0.029	0.023	0.025
3	0.304	0.291	0.298	0.298
4	0.612	0.594	0.597	0.601
5	1.080	1.140	1.137	1.119
6	0.021	0.006	0.018	0.015
7	0.025	0.043	0.018	0.029
8	0.038	0.020	0.018	0.025
9	0.030	0.012	0.013	0.018
10	0.151	0.108	0.174	0.144
11	0.146	0.147	0.139	0.144

Table 1: Activity test of MP-19

A1-D1: Phosphate standard O pmol
A2-D2: Phosphate standard 100 pmol
A3-D3: Phosphate standard 500 pmol
A4-D4: Phosphate standard 1000 pmol
A5-D5: Phosphate standard 2000 pmol
A6-D6: mouse dhfr gene with substrate (negative control)
A7-D7: mouse dhfr gene without substrate (negative control)
A8-D8: MP-19 with modified PPTase-2C buffer (5mM $MgCl_2$ is replaced by 5 mM $CaCl_2$) and substrate
A9-D9: MP-19 with PPTase-2C buffer without substrate
A10-D10: MP-19 with PPTase-2C buffer and substrate
A11-D11: MP-19 with PPTase-2C buffer, substrate and 10 µM okadaic acid

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Biopharm GmbH
(B) STREET: Czernyring 22
(C) CITY: Heidelberg
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 69115

(ii) TITLE OF INVENTION: Nucleic acid encoding a novel human protein phosphatase

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 98107346.3

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 678 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TACGGGCAGA ACTGTCACAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG GACAGGCGAG 60

GAACCAGGGT CCCAGGGCCT CAATGGGGAG GCAGGACCTG AGGACTCAAC TAGGGAAACT 120

CCTTCACAAG AAAATGGCCC CACAGCCAAG GCCTACACAG GCTTTTCCTC CAACTCGGAA 180

CGTGGGACTG AAGCAGGCCA AGTTGGTGAG CCTGGCATTC CCACTGGTGA GGCTGGGCCT 240

TCCTGCTCTT CAGCCTCTGA CAAGCTGCCT CGAGTTGCTA AGTCCAAGTT CTTTGAGGAC 300

AGTGAGGATG AGTCAGATGA GGCGGAGGAA GAAGAGGAAG ACAGTGAGGA ATGCAGCGAG 360

GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG AAGATGAGGA TGACACCGAG 420

GAGGCTGAAG AGGACGATGA AGAAGAAGAA GAAGAGATGA TGGTGCCAGG GATGGAAGGC 480

AAAGAGGAGC CTGGCTCTGA CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG 540

CAGTTGATTG TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAGCT 600

TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCACG CATCAAGAAT 660

GCTGGTGGCA AGGTCACC 678

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 226 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Tyr Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly
1 5 10 15

Gly Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly
20 25 30

Pro Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr
35 40 45

Ala Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu

```
     50                  55                  60

Ala Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro
65                  70                  75                  80

Ser Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys
                85                  90                  95

Phe Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu Glu
            100                 105                 110

Glu Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu
        115                 120                 125

Glu Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu
    130                 135                 140

Asp Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly
145                 150                 155                 160

Lys Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu
                165                 170                 175

Ile Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys
            180                 185                 190

Val Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys
        195                 200                 205

Pro Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys
    210                 215                 220

Val Thr
225
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1641 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGGTGCCT ACCTCTCCCA GCCCAACACG GTGAAGTGCT CCGGGGACGG GGTCGGCGCC 60

CCGCGCCTGC CGCTGCCCTA CGGCTTCTCC GCCATGCAAG GCTGGCGCGT CTCCATGGAG 120

GATGCTCACA ACTGTATTCC TGAGCTGGAC AGTGAGACAG CCATGTTTTC TGTCTACGAT 180

GGACATGGAG GGGAGGAAGT TGCCTTGTAC TGTGCCAAAT ATCTTCCTGA TATCATCAAA 240

GATCAGAAGG CCTACAAGGA AGGCAAGCTA CAGAAGGCTT TAGAAGATGC CTTCTTGGCT 300

ATTGACGCCA AATTGACCAC TGAAGAAGTC ATTAAAGAGC TGGCACAGAT TGCAGGGCGA 360

CCCACTGAGG ATGAAGATGA AAAAGAAAAA GTAGCTGATG AAGATGATGT GGACAATGAG 420

GAGGCTGCAC TGCTGCATGA AGAGGCTACC ATGACTATTG AAGAGCTGCT GACACGCTAC 480

GGGCAGAACT GTCACAAGGG CCCTCCCCAC AGCAAATCTG GAGGTGGGAC AGGCGAGGAA 540

CCAGGGTCCC AGGGCCTCAA TGGGGAGGCA GGACCTGAGG ACTCAACTAG GGAAACTCCT 600

TCACAAGAAA ATGGCCCCAC AGCCAAGGCC TACACAGGCT TTTCCTCCAA CTCGGAACGT 660

GGGACTGAGG CAGGCCAAGT TGGTGAGCCT GGCATTCCCA CTGGTGAGGC TGGGCCTTCC 720

TGCTCTTCAG CCTCTGACAA GCTGCCTCGA GTTGCTAAGT CCAAGTTCTT TGAGGACAGT 780

GAGGATGAGT CAGATGAGGC GGAGGAAGAA GAGGAAGACA GTGAGGAATG CAGCGAGGAA 840

GAGGATGGCT ACAGCAGTGA GGAGGCAGAG AATGAGGAAG ATGAGGATGA CACCGAGGAG 900

GCTGAAGAGG ACGATGAAGA AGAAGAAGAA GAGATGATGG TGCCAGGGAT GGAAGGCAAA 960

GAGGAGCCTG GCTCTGACAG TGGTACAACA GCGGTGGTGG CCCTGATACG AGGGAAGCAG 1020

TTGATTGTAG CCAACGCAGG AGACTCTCGC TGTGTGGTAT CTGAGGCTGG CAAAGCTTTA 1080

GACATGTCCT ATGATCACAA ACCAGAGGAT GAAGTAGAAC TAGCACGCAT CAAGAATGCT 1140

GGTGGCAAGG TCACCATGGA TGGGCGAGTC AACGGGGGCC TCAACCTCTC CAGAGCCATT 1200

GGGGACCACT TCTATAAGAG AAACAAGAAC CTGCCACCTG AGGAACAGAT GATTTCAGCC 1260

CTTCCTGACA TCAAGGTGCT GACTCTCACT GACGACCATG AATTCATGGT CATTGCCTGT 1320

GATGGCATCT GGAATGTGAT GAGCAGCCAG GAAGTTGTAG ATTTCATTCA ATCAAAGATC 1380

AGCCAGCGTG ATGAAAATGG GGAGCTTCGG TTATTGTCAT CCATTGTGGA AGAGCTGCTG 1440

GATCAGTGCC TGGCACCAGA CACTTCTGGG GATGGTACAG GGTGTGACAA CATGACCTGC 1500

ATCATCATTT GCTTCAAGCC CCGAAACACA GCAGAGCTCC AGCCAGAGAG TGGCAAGCGA 1560

AAACTAGAGG AGGTGCTCTC TACTGAGGGG GCTGAAGAAA ATGGCAACAG CGACAAGAAG 1620

AAGAAGGCCA AGCGAGACTA G 1641

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Ala Tyr Leu Ser Gln Pro Asn Thr Val Lys Cys Ser Gly Asp
1 5 10 15

Gly Val Gly Ala Pro Arg Leu Pro Leu Pro Tyr Gly Phe Ser Ala Met
20 25 30

Gln Gly Trp Arg Val Ser Met Glu Asp Ala His Asn Cys Ile Pro Glu
35 40 45

Leu Asp Ser Glu Thr Ala Met Phe Ser Val Tyr Asp Gly His Gly Gly
50 55 60

Glu Glu Val Ala Leu Tyr Cys Ala Lys Tyr Leu Pro Asp Ile Ile Lys
65 70 75 80

Asp Gln Lys Ala Tyr Lys Glu Gly Lys Leu Gln Lys Ala Leu Glu Asp
85 90 95

Ala Phe Leu Ala Ile Asp Ala Lys Leu Thr Thr Glu Glu Val Ile Lys
100 105 110

Claims

1. A nucleic acid comprising a nucleotide sequence encoding a human serine/threonine phosphatase or a functional fragment thereof that is capable of dephosphorylating serine or threonine residues, wherein the nucleotide sequence comprises:

(a) the nucleotides as shown in SEQ ID NO. 1 or SEQ ID NO. 3; or

(b) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequence of (a); or

(c) an allelic derivative of the sequences of (a) or (b); or

(d) a DNA sequence which is capable of hybridizing to the sequences in (a), (b) and (c), and encoding a protein containing the amino acid sequence as depicted in SEQ ID NO. 2 or SEQ ID NO. 4; or

(e) a nucleotide sequence which is capable of hybridizing to the DNA sequences in (a), (b), (c) and (d), and encoding a protein having essentially the same biological properties as the protein defined in (d).

2. The nucleic acid according to claim 1, wherein the nucleotide sequence is a vertebrate DNA sequence, a mammalian sequence, preferably a primate, human, porcine, or rodent, preferably a rat or rabbit, DNA sequence.

3. A recombinant molecule comprising a nucleic acid according to claim 1 or 2.

4. The recombinant molecule according to claim 3, wherein said nucleic acid sequence is functionally linked to an expression-control sequence.

5. A host containing the nucleic acid according to claim 1 or 2, or the recombinant molecule according to claim 3 or 4.

6. The host according to claim 5, which is a bacterium, a fungus, a plant cell, an animal or a human cell.

7. A process for the production of a PP2C-like protein comprising cultivating a host according to claim 5 or 6 and recovering said PP2C-like protein from the culture.

8. A PP2C-like protein or a biologically active fragment thereof encoded by a nucleic acid according to claim 1 or 2 or by a recombinant molecule according to claim 3 or 4.

9. The protein according to claim 8, comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4.

10. An agonist as a substitute for the protein of claim 8 or 9.

11. An antagonist directed to the protein of claim 8 or 9.

12. A pharmaceutical composition containing the protein or a biologically active fragment thereof according to claim 8 and 9 or the agonist according to claim 10 or the antagonist according to claim 11, and optionally, a pharmaceutically acceptable carrier and/or diluent.

13. The pharmaceutical composition according to claim 12 for the treatment of leukemia, brain cancer, breast cancer, prostate cancer, Alzheimer's disease, Huntingdon's disease, Parkinson's disease, and epilepsy, and of disorders of the reproductive system, or for the regulation of spermatogenesis or the maturation of mammalian germ cells.

14. An antibody or antibody fragment which is capable of specifically binding to the protein of claim 8 and 9 or to the agonist of claim 10 or to the antagonist of claim 11.

15. The antibody according to claim 14, which is a monoclonal antibody.

16. Use of an antibody or antibody fragment according to claim 14 or 15 for detecting the protein or a biologically active fragment thereof as defined in claim 8 or 9.

17. A diagnostic kit containing the agonist according to claim 10 or the antagonist according to claim 11 or the antibody or antibody fragment according to claim 14 or 15.

Fig. 1

```
MP19-PCR     YGQNCHKGPP HSKSGGGTGE EPGSQGLNGE AGPEDSTRET PSQENGPTAK   50
PP2C-Human   MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
PP2C-Rabbit  MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
PP2C-Rat     MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
              *    *          * *       **          *

MP19-PCR     AYTGFSSNSE RGTEAGQVGE PGIPTGEAGP SCSSASDKLP RVAKSKFFED  100
PP2C-Human   LPSGLESWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
PP2C-Rabbit  LPSGLETWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
PP2C-Rat     LPSGLETWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
                 *       *           *   *                    *          *             *

MP19-PCR     SEDESDEAEE EEEDSEECSE EEDGYSSEEA ENEEDEDDTE EAEEDDEEEE  150
PP2C-Human   SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID  109
PP2C-Rabbit  SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID  109
PP2C-Rat     SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID  109
              *           *                        *                              *

MP19-PCR     EEMMVPGMEG KEEPGSDSGT TAVVALIRGK QLIVANAGDS RCVVSEAGKA  200
PP2C-Human   EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV  157
PP2C-Rabbit  EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV  157
PP2C-Rat     EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV  157
              * * *   *     *          **   ***   **               * *** *          *

MP19-PCR     LDMSYDHKPE DEVELARIKN AGGKVT                            226
PP2C-Human   HFFTQDHKPS NPLEKERIQN AGGSVM                            183
PP2C-Rabbit  HFFTQDHKPS NPLEKERIQN AGGSVM                            183
PP2C-Rat     HFFTQDHKPS NPLEKERIQN AGGSVM                            183
                  ****     *  ** * *** *
```

Figure 2

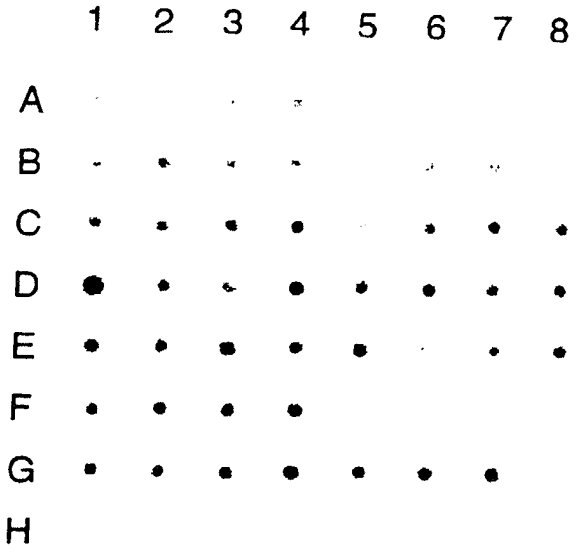
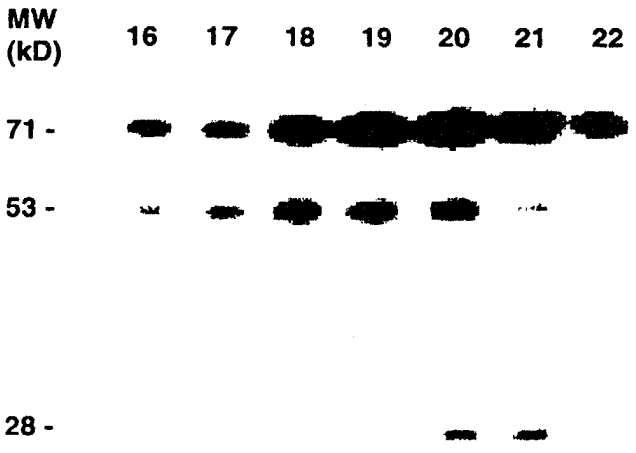


Figure 3



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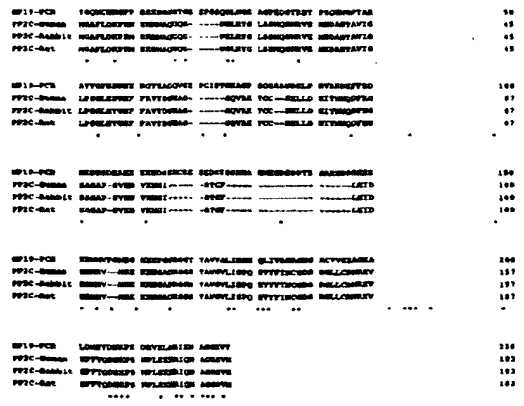
Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Nucleic acid encoding a human protein phosphatase

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Fig. 1



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Description

The present invention relates to nucleic acids encoding a novel human protein phosphatase of the protein serine/threonine phosphatase family. In particular, it relates to novel DNA sequences encoding a serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Protein phosphorylation-dephosphorylation is a universal mechanism by which different cellular events are regulated. The serine/threonine-specific phosphatases have been classified into four main types according to their in vitro specificity for selected substrates and sensitivity to activators and inhibitors (Ingebritsen, T.S. and Cohen, P. (1983) Eur. J. Biochem. 182, 255-261). Sequence analysis revealed that they can be classified into two major gene families. The first one includes type 1 (PP1), type 2A (PP2A), and type 2B (PP2B) phosphatases, which share 37 to 59 % sequence identity (Barton, G.J. et al., (1994) Eur. J. Biochem. 220, 225-237) in their catalytic domains and are inhibited by okadaic acid (Bialojan, C., and Takai, A. (1988) Biochem. J. 256, 283-290). The second family, the Mg^{2+}-dependent phosphatases, also designated type 2C (PP2C), share little sequence similarity with the first family and are insensitive to okadaic acid. cDNA sequences of PP2C α and β from mammalian sources showed > 90 % identity. PP2Cs have been implicated in the regulation of fatty acid and cholesterol biosynthesis (Moore, F. et al. (1991) Eur. J. Biochem. 199, 691-697) and heat shock response (Maeda et al. (1993) Mol. Cell. Biol. 113, 5408-5417, Shiozaki, K. et al. (1994) Mol. Cell. Biol. 14, 3742-375).

The technical problem underlying the present invention is to provide a new human PP2C-like protein phosphatase which is distinct from the other PP2Cs.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims. Other features and advantages of the invention will be apparent from the description of the preferred embodiments and drawings.

The sequence listings and drawings will now be briefly described.

SEQ ID NO. 1 shows the nucleotide sequence of MP-19, a DNA sequence derived from human placenta.

SEQ ID NO. 2 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 1.

SEQ ID NO. 3 shows the nucleotid sequence of MP-19 full-lenght cDNA derived from human placenta.

SEQ ID NO. 4 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 3.

The figures show:

Figure 1 shows the alignment of the amino acid sequence of MP-19 with some related proteins of the PP2C family. The asterisk (*) indicates the position of identical amino acids of the compared amino acid sequences.

MP19-PCR:	amino acid sequence of MP-19 (SEQ ID NO. 2)
PP2C-Human:	human protein phosphatase 2C alpha (Accession No. S87759)
PP2C-Rabbit:	rabbit protein phosphatase 2C alpha (Accession No. S87757)
PP2C-Rat:	rat protein phosphatase 2C (Accession No. J04503)

Figure 2 is a human RNA master blot. The RNA-dot blot analysis shows hybridization of MP-19 PCR probe with different human RNA samples.
A1 whole brain, A2 amygdala, A3 caudate nucleus, A4 cerebellum, A5 cerebral cortex, A6 frontal lobe, A7 hippocampus, A8 medulla oblongata, B1 occipital lobe, B2 putamen, B3 substantia nigra, B4 temporal lobe, B5 thalamus, B6 subthalamic nucleus, B7 spinal cord, B8 blank, C1 heart, C2 aorta, C3 skeletal muscle, C4 colon, C5 bladder, C6 uterus, C7 prostate, C8 stomach, D1 tests, D2 ovary, D3 pancreas, D4 pituitary gland, D5 adrenal gland, D6 thyroid gland, D7 salivary gland, D8 mammary gland, E1 kidney, E2 liver, E3 small intestine, E4 spleen, E5 thymus, E6 peripheral leukocyte, E7 lymph node, E8 bone marrow, F1 appendix, F2 lung, F3 trachea, F4 placenta, F5-F8 blank, G1 fetal brain, G2 fetal heart, G3 fetal kidney, G4 fetal liver, G5 fetal spleen, G6 fetal thymus, G7 fetal lung, G8 blank, H1 yeast total RNA, H2 yeast tRNA, H3 *E. coli* rRNA, H4 *E. coli* DNA, H5 poly r(A), H6-H8 human DNA.

Figure 3 shows the detection of MP-19 after IMAC. The Western analysis was performed after purification of MP-19 using Immobilized Metal Ion Affinity Chromatography (IMAC). Positive signals were obtained from fraction 16 to fraction 22.

The amino acid sequence alignment of MP-19 with sequences of different PP2Cs (shown in Figure 1) demonstrates the homology of MP-19 to the PP2C family but implicate also that MP-19 belongs to a new protein phosphatase group. The homology of the derived MP-19 amino acid sequence (aa 1 - aa 226) to PP2C from human, rabbit and rat displays a sequence homology of 21.2 %. Moreover a partial sequence of MP-19 (amino acid sequence 158 - 226) which is in the shown alignment not disrupted by gaps indicates a sequence homology of 39.1 % to the compared PP2Cs.

The present invention relates particularly to a novel serine/threonine protein phosphatase and, preferably, provides DNA sequences contained in the corresponding gene.

Such sequences include nucleotide sequences as illustrated in SEQ ID NO. 1 and SEQ ID NO. 3, allelic derivatives of said sequences and DNA sequences degenerated as a result of the genetic code for said sequences. It also includes DNA sequences hybridizing under stringent conditions with the DNA sequence mentioned above. It further includes antisense nucleic acid, preferably antisense MP-19 nucleic acid, directed to the above defined nucleic acid. The terms "nucleic acid sequence" and "nucleotide sequence" refers to DNA or RNA or heterooligomeric sequences, which may be double- or single-stranded.

Although said allelic, degenerate and hybridizing sequences may have structural divergences due to naturally occurring mutations, such as small deletions or substitutions, they will usually still exhibit essentially the same useful properties, allowing their use in basically the same medical or diagnostic applications.

According to the present invention, the term "hybridization" means conventional hybridization conditions, preferably conditions with a salt concentration of 6 x SSC at 62°C to 66°C followed by a one-hour wash with 0.6 x SSC, 0.1% SDS at 62°C to 66°C.

Important biological embodiments of the present invention are DNA sequences of the above and obtainable from vertebrates, preferably mammals such as pig and from rodents such as rat, and in particular from primates such as humans.

Particularly preferred embodiments of the present invention are the DNA sequence termed MP-19 which are shown in SEQ ID NO. 1 and SEQ ID No. 3. The corresponding transcripts of MP-19 were obtained from human placenta tissue and code for a protein showing considerable amino acid homology to the PP2C proteins (shown in Figure 1 deduced from SEQ ID NO. 1). The protein sequence of rabbit and human PP2C α and rat and rabbit PP2C α are described in Mann et al. (1992) Biochim. Biophys. Acta 1130, 100-104. Some typical sequence homologies, which are specific for the known PP2Cs, were also found in the MP-19 sequence. In the present invention, cloning was carried out according to the method described below. Once the DNA sequence has been cloned, the preparation of host cells capable of producing the PP2C-like protein MP-19 and the production of said protein can be easily accomplished using known recombinant DNA techniques comprising constructing the expression plasmids encoding said protein and transforming a host cell with said expression plasmid, cultivating the transformant in a suitable culture medium, and recovering the product having PP2C-like activity.

Thus, the invention also relates to recombinant molecules comprising DNA sequences as described above, optionally linked to an expression control sequence. Such vectors may be useful in the production of the PP2C-like protein in stable or transiently transformed cells. Several animal, plant, fungal and bacterial systems may be employed for the transformation and subsequent cultivation process. Preferably, expression vectors which can be used in the invention contain sequences necessary for the replication in the host cell and are autonomously replicable. It is also preferable to use vectors containing selectable marker genes which can be easily selected for transformed cells. The necessary operation is well-known to those skilled in the art.

It is another object of the invention to provide a host cell transformed by an expression plasmid of the invention and capable of producing a protein of the serine/threonine phosphatase family. Examples of suitable host cells include various eucaryotic and procaryotic cells, such as *E. coli*, insect cells, plant cells, mammalian cells, and fungi such as yeast.

Another object of the present invention is to provide a PP2C-like protein or a biologically active fragment thereof encoded by the sequences described above and displaying biological features such as dependency of Mg^{2+} (or Mn^{2+}) for activity. Furthermore, the phosphatase catalyzes dephosphorylation of phosphoseryl/threonyl residues of proteins and peptides phosphorylated by cAMP-dependent protein kinases and protein kinase C. It is insensitive to inhibitors like okadaic acid and calyculin A, heparin and PP1 inhibitors 1 and 2. It does not attack phosphorylase α. It is inhibited by polycations and F^- ions. A preferred substrate for the PP2C-like protein is the SET protein, suggesting capacities possible relevant to therapeutically treatment of leukemia. Furthermore, the PP2C-like protein prefers basic substrates such as histones, and MBP phosphorylated by cAMP-dependent protein kinase, suggesting a special function for this phosphatase in the brain. The amino acid sequences of especially preferred PP2C-like proteins (MP-19) are shown in SEQ ID NO. 2 and SEQ ID NO. 4.

It is a further aspect of the invention to provide a process for the production of PP2C-like proteins. Such a process comprises cultivating a host cell being transformed with a nucleic acid sequence of the present invention in a suitable culture medium and purifying the PP2C-like protein produced. Thus, this process allows the production of the sufficient amount of the desired protein for use in medical treatments. The host cell is obtainable from bacteria such as *Bacillus spec.* or *Escherichia coli*, from fungi such as yeast, from plants such as tobacco, potato, or *Arabidopsis*, and from animals, in particular vertebrate cell lines such as the Mo-, COS- or CHO cell line.

It is another object of the present invention to provide pharmaceutical compositions containing a therapeutically-effective amount of a PP2C-like protein of the present invention and, optionally, a pharmaceutically acceptable carrier and/or diluent, and/or agonists and/or antagonists thereof. Such a therapeutic composition can be used for the treatment of cancer such as leukemia, brain cancer, breast cancer and prostate cancer. The pharmaceutical composition according to the present invention can also be therapeutically applied for degenerative disorders of the CNS, e.g. Alzheimer's disease, Huntingdon's disease, Parkinson's disease, and epilepsy, and disorders of the reproductive system e.g. fertility disorders or testicular cancer. Another possible clinical application of a PP2C-like protein is the use for treatment of liver diseases, diabetes, and cystic fibrosis. The pharmaceutical composition comprising the protein of the present invention can also be used for treatment of microbial or viral infections. Another application of the pharmaceutical composition of the present invention is the usage of the protein in the regulation of spermatogenesis or the maturation of mammalian germ cells, e.g. for centraception.

Furthermore, the application of the composition is not limited to humans but can include animals, in particular domestic animals, as well.

Finally, another object of the present invention is an antibody or antibody fragment, which is capable of specifically binding to the proteins of the present invention. Methods to raise such specific antibody are known in the art. Such an antibody is preferably a monoclonal antibody. Such antibodies or antibody fragments might be useful for diagnostic methods.

The following examples illustrate in more detail the present invention, but should not be construed as limiting the invention.

Example 1

Isolation of MP-19

For the reverse transcription reaction, 5 µg total RNA (0.5 µg/µl) derived from human placenta tissue was heated for 5 minutes and cooled rapidly on ice for 5 minutes. The reverse transcription reagent mixture containing 5 µg total RNA, 38 u of RNA-guard (Pharmacia), 2.5 µg oligomer d(T)12-18 (Boehringer Mannheim), 5x reaction buffer (250 mM Tris/HCl pH 8.5; 50 mM $MgCl_2$; 50 mM DTT; 600 mM KCl), 10 mM of each dNTP (Pharmacia), 37.5 u of avian myoblastosis virus reverse transcriptase (AMV, Boehringer Mannheim). The reaction mixture (20 µl) was incubated for 90 minutes at 42°C. The resulting placenta cDNA pool was stored at -20°C.

For the primary polymerase chain reaction (PCR), a placenta-derived cDNA pool was used as template in a 50 µl reaction mixture. The PCR reaction was carried out in a RoboCycler Gradient 96 (Stratagene). The amplification was performed in 1x PCR-buffer (10 mM Tris/HCl pH 8.3; 50 mM KCl; 0.001 % gelatine), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide (ALK6-N2, 5' - TT(CT)(AG)C(AGCT)AT(AGCT)ATAGAAGAAGATGA - 3' and ALK6-R2, 5' - CC(AGCT)CGCCA(CT)TT(AGCT)CCCATCCA - 3') and 1.5 u Taq polymerase (Perkin Elmer). The PCR reaction contained cDNA corresponding to 30 ng of total RNA as starting material. The reaction mixture was overlaid by 40 µl paraffin incubated for 180s/94°C and subjected to 30 cycles (50s/94°C, 90s/48 °C, 60s/72 °C) with an additional extension for 480s/72 °C in the Thermocycler.

A second round of amplification was performed as described above with exception that 5 µl from the first PCR reaction was used as template DNA for the PCR. A 10 µl sample from the second PCR amplification was fractionated by electrophoresis using a 2 % agarose gel in TBE buffer. After electrophoresis amplified DNA corresponding to a molecular weight of about 600-800 bp was excised from the gel and isolated by 3x freeze/thaw cycles (-20 °C/ + 37 °C) and using the DNA Purification Kit "Easy Pure" (Biozyme, Cat. no. 39001) following the instructions of the manufacturer.

The eluted DNA was amplified a third time as described in the primary PCR with exception that 3 µl of the eluted DNA, resulted from the second round of amplification, was used as template for PCR and the annealing temperature was 56 °C instead of 48 °C. After electrophoresis using a 2% agarose gel in TBE buffer, a distinct DNA band that corresponds to a molecular weight of about 700 bp was eluted with the extraction method described before. After than an additionally purification using the QIAquick 8 PCR Purification Kit (Qiagen, Cat. no. 28144) following the instructions of the manufacturer, was carried out.

Cloning of the purified DNA was established using the Original TA Cloning Kit (Invitrogen, Cat. no. K2000-40). Plasmid DNA from positive clones was isolated with the QIAwell 8 Plus Plasmid Kit (Qiagen, Cat. no. 16142) and sequenced with an automatic DNA sequencer (ALFexpress, Pharmacia). The resulting DNA sequence was analyzed by a homol-

ogy search with the blast program.

Example 2

Isolation of MP-19 full-length cDNA

Isolation of a full-length cDNA clone of MP-19 was performed with a commercial available Human Placenta Lambda cDNA Library (Stratagene, Cat. no. 937225). For screening, a labeled PCR probe was generated from MP-19 DNA (SEQ ID NO. 1). The amplification was performed in 1x PCR-buffer (Qiagen, Germany), 1 mM of dATP, 1 mM of dCTP, 1 mM of dGTP, 0.6 mM of dTTP (Pharmacia, Germany), 0.4 mM of Digoxigenin-11-dUTP (Boehringer, Mannheim), 100 pmol of each oligonucleotide PL19-N1 (5'-GGGCAGAACTGTCACAAGGG-3') and PL19-R1 (5'-CATCCATGGTGACCTTGCCACC-3') and 1 u Taq DNA-polymerase (Qiagen). The PCR mix was overlaid by 40 µl paraffin, incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/58 °C, 60s/72 °C) with an additional extension for 180s/72 °C.

Prehybridization of plaque lift filters from cDNA library were done at 58 °C for 4 h in 0.25 M Na_2HPO_4, 7 % SDS, 1 % BSA, 1 mM EDTA, pH 7.2. Hybridization was carried out with 50 ng labeled MP19 PCR probe for 15 h under same buffer conditions as prehybridization was done. Filter washed 3 times (5 min, 10 min and 15 min) with 30 mM Na_2HPO_4, 0,1 % SDS at 60 °C. Detection of signals were performed with DIG Luminescent detection kit from Boehringer, Mannheim (Cat. no. 1363514). Proceed from a positive signal, clone 39-1 was isolated and sequenced. The resulting DNA-sequence from clone 39-1 conform to Bp. 387-1641 in SEQ ID NO. 3. To generate the 5'cDNA end of MP-19 full-lenght cDNA, 1 µg of a Lambda-DNA preparation from Human Placenta Lambda cDNA Library was subjected to PCR. The ampilfication was performed in 1x PCR-buffer (Qiagen), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucieotide MP19-E5 (5'-GGATCCATGGGTGCCTACCTCTCCCAGCCC-3') which was derived from an EST sequence (accession no. AA115688) and MP19-3 (5'-GCCTGTGTAGGCCTTGGCTGTGGGGCC-3') and 1 u Taq DNA-polymerase (Qiagen). The reaction was overlaid by 40 µl paraffin incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/74 °C, 60s/72 °C) with an additional extension for 300s/72 °C. The resulting PCR fragment was subcloned in vector pCR 2.1 (Clontech, Germany). Corresponding DNA was digested with restriction endonucleases Bam HI and Stu I. After that MP-19 Bam HI/Stu I fragment was inserted into clone 39-1. The resulted clone was named 28-9 which DNA-sequence present the full-lenght cDNA-sequence of MP-19 as shown in SEQ ID NO. 3. The corresponding amino acid sequence of MP-19 is shown in SEQ ID NO. 4.

Gene expression of MP-19 in human tissues

Relative expression of the MP-19 gene was determined by Northern blot analyses. A commercial available Human RNA Master Blot (Clontech, Germany, Cat. no. 7770-1, Lot no. 7090716) was hybridized with the digoxigenin labeled MP-19 PCR probe as described in: Isolation of MP-19 full-length cDNA. 50 different human tissues samples were investigated for MP-19 gene expression. Additionally 8 different negative controls from *E. coli*, yeast and human genomic DNA were applied.

Main expression of MP-19 was detected in human testis which is shown in figure 2. Lower expression of MP-19 was detected in human pituitary gland, thymus, small intestine and fetal liver. Basal expression of MP-19 was found in all other human RNA samples. No hybridization signals were detected in negative controls.

Expression of MP-19 cDNA in *E. coli*

The cDNA of clone 28-9 was subcloned into the expression plasmid pQE-16 (Qiagen, Germany). This cloning strategy constituted an additional tag of 6 histidine residues at the C-terminus of MP-19. pQE-16 was digested with Bam HI and Bgl II. The 5' part of MP-19 was excised from clone 28-9 with Bam HI and Sac I. To constitute a compatible 3'end of MP-19 for cloning into plasmid pQE-16, a PCR was performed with primer MP19N-Sac I (5'-ACAGCAGAGCTCCAGCCAGAG-3') and MP19R-Bgl II (5'-AGATCTGTCTCGCTTGGCCTTCTTCTTC-3') and template DNA of clone 28-9.5' and 3'end of MP-19 DNA was ligated into pQE-16 to establish MP-19 with His-tag, which was expressed in *E. coli* strain M15 (Qiagen). For recombinant expression of MP-19, cells were grown in a 5 l fermenter (Bio Console ADI 1035, Applikon, Netherlands) at 37 °C in LB-Medium until an OD_{600} of 2,5 was reached. After induction with 1 mM β-D-thiogalactopyranoside, cells were grown for additional 4 h until OD_{600} of 9,7 was reached. Cells were harvested by centrifugation at 10.000 x g for 30 min, washed once in 500 ml 1 x PBS buffer (30 min at 10.000 x g) and were frozen in aliquots at -80 °C. For preparation of MP-19 protein, 10 g cells were lysed in 100 ml lysis buffer (50 mM NaH_2PO_4, pH 8,0, 300 mM NaCl, 10 mM imidazole, 100 mg lysozyme (Serva, Germany) and 50 u Benzonase (Merck, Germany)) following by sonication 3 times with a ultrasonic processor (UP-200S, Dr. Hielscher GmbH, Germany) for 3 min at 5 $kWsec^{-1}$ in an ice/water bath. Cell debris was removed by centrifugation for 30 min at 4 °C and 25.000 x g.

Purification of recombinant MP-19

Recombinant expressed MP-19 was purified using Immobilized Metal Ion Affinity Chromatography (IMAC) and Reverse Phase Liquid Chromatography (RPLC). The chromatographic purification was realized using the ÄKTA-Explorer system (Pharmacia Biotech, Germany). For IMAC, a 1 ml Hi-Trap cheating column (Pharmacia Biotech) is used. Hi-Trap column was activated with 5 column volumes (cv) of 100 mM $NiSO_4$, afterwards the column washed with 5 cv water to remove unbound Ni^{2+}. Column equilibration was performed with 5 cv of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazole, pH 8,0). Cell lysat results from 1 g *E. coli* cells was loaded onto column. Afterwards the column washed with lysis buffer to remove unbound protein. Protein were eluted using the following gradient program. Step 1: 20 mM imidazole to 300 mM imidzole within 20 minutes, step 2: 300 mM imidazol to 500 mM imidazol within 10 minutes using buffer A (50 mM Tris, 300 mM NaCl, 20 mM Imidzole, pH 6,0) and buffer B (50 mM Tris, 300 mM NaCl, 500 mM Imidazole, pH 6,0). Flow rate of chromatography were 1 ml/min, detection were performed at 280 nm. Fractions were analyzed by immunological detection, shown in Figure 3. For further purification, positive fractions containing MP-19 were pooled and loaded onto a Resource RPC (3ml) column (Pharmacia Biotech). Column was equilibrated with buffer A (0.1 % trifluor acetic acid) and protein eluted with a linear gradient of buffer B (0,1 % TFA-90 % acetonitrile). Flow rate of chromatography were 3 ml/min, detection were performed at 215 nm.

Immunolonical Detection of MP-19

Immunological detection of recombinant MP-19 was performed by western blotting using a commercial available monoclonal mouse antibody against histidine-tag (Dianova, Germany, Cat. no. dia900) in combination with Western Light chemoluminescent detection system using the goat anti-mouse-AP antibody (Tropix, U.S.A.).

Activity assay for recombinant MP-19

A Serine/Threonine Phosphatase Assay System (Promega, Germany, Cat. no. V2460) was used to determine enzymatic activity of recombinant MP-19. This assay use a chemically synthesized phosphopeptide RRA(pT)VA which is a functional substrate for MP-19 phosphatase. The amount of free phosphate which is generated by MP-19 enzymatic reaction was measured by the absorbency of a molybdate:malachit green:phosphate complex (Ekman P. and Jager O. (1993), Anal. Biochem. 214, 138-141, Deana A. D. et al. (1990), Biochimica et Biophysica Acta 1051, 199-202). Assays were performed as described by the manufacturer in PPTase-2C buffer (50 mM imidazole, pH 7,2, 0,2 mM EGTA, 5 mM $MgCl_2$, 0.02 % β-mercaptoethanol, 0,1 mg/ml BSA). To determine background of this assay, clone pQE-16-dhfr (Qiagen, Germany) was used, which is identical to pQE-MP-19 with exception that vector pQE-16 inserted a mouse dhfr gene instead of MP-19 phosphatase gene.

Results of activity assay of MP-19 shown in table 1. MP-19 has a significant activity in a $MgCl_2$ containing buffer, but no activity in a $CaCl_2$ containing buffer, which shows the requirement to Mg^{2+}. Inhibitors like okadaic acid (10 µM) shows no significant reduction of MP-19 activity. Control expression of the mouse dhfr gene shows no activity in the Serine/Threonine Phosphatase Assay System.

	A	B	C	D (Average A-C)
1	0.000	0.006	-0.006	0.000
2	0.024	0.029	0.023	0.025
3	0.304	0.291	0.298	0.298
4	0.612	0.594	0.597	0.601
5	1.080	1.140	1.137	1.119
6	0.021	0.006	0.018	0.015
7	0.025	0.043	0.018	0.029
8	0.038	0.020	0.018	0.025
9	0.030	0.012	0.013	0.018
10	0.151	0.108	0.174	0.144
11	0.146	0.147	0.139	0.144

Table 1: Activity test of MP-19

A1-D1: Phosphate standard O pmol
A2-D2: Phosphate standard 100 pmol
A3-D3: Phosphate standard 500 pmol
A4-D4: Phosphate standard 1000 pmol
A5-D5: Phosphate standard 2000 pmol
A6-D6: mouse dhfr gene with substrate (negative control)
A7-D7: mouse dhfr gene without substrate (negative control)
A8-D8: MP-19 with modified PPTase-2C buffer (5mM $MgCl_2$ is replaced by 5 mM $CaCl_2$) and substrate
A9-D9: MP-19 with PPTase-2C buffer without substrate
A10-D10: MP-19 with PPTase-2C buffer and substrate
A11-D11: MP-19 with PPTase-2C buffer, substrate and 10 μM okadaic acid

Annex to the description

Sequence listing

SEQ ID NO. 1

```
TACGGGCAGA ACTGTCACAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG      50
GACAGGCGAG GAACCAGGGT CCCAGGGCCT CAATGGGGAG GCAGGACCTG     100
AGGACTCAAC TAGGGAAACT CCTTCACAAG AAAATGGCCC CACAGCCAAG     150
GCCTACACAG GCTTTTCCTC CAACTCGGAA CGTGGGACTG AAGCAGGCCA     200
AGTTGGTGAG CCTGGCATTC CCACTGGTGA GGCTGGGCCT TCCTGCTCTT     250
CAGCCTCTGA CAAGCTGCCT CGAGTTGCTA AGTCCAAGTT CTTTGAGGAC     300
AGTGAGGATG AGTCAGATGA GGCGGAGGAA GAAGAGGAAG ACAGTGAGGA     350
ATGCAGCGAG GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG     400
AAGATGAGGA TGACACCGAG GAGGCTGAAG AGGACGATGA AGAAGAAGAA     450
GAAGAGATGA TGGTGCCAGG GATGGAAGGC AAAGAGGAGC CTGGCTCTGA     500
CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG CAGTTGATTG     550
TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAGCT     600
TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCACG     650
CATCAAGAAT GCTGGTGGCA AGGTCACC                             678
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SEQ ID NO. 2

```
YGQNCHKGPP HSKSGGGTGE EPGSQGLNGE AGPEDSTRET PSQENGPTAK      50
AYTGFSSNSE RGTEAGQVGE PGIPTGEAGP SCSSASDKLP RVAKSKFFED     100
SEDESDEAEE EEEDSEECSE EEDGYSSEEA ENEEDEDDTE EAEEDDEEEE     150
EEMMVPGMEG KEEPGSDSGT TAVVALIRGK QLIVANAGDS RCVVSEAGKA     200
LDMSYDHKPE DEVELARIKN AGGKVT                               226
```

SEQ ID NO. 3

ATGGGTGCCTACCTCTCCCAGCCCAACACGGTGAAGTGCTCCGGGGACGGGGTCGGCGCCCC
GCGCCTGCCGCTGCCCTACGGCTTCTCCGCCATGCAAGGCTGGCGCGTCTCCATGGAGGATG
CTCACAACTGTATTCCTGAGCTGGACAGTGAGACAGCCATGTTTTCTGTCTACGATGGACAT
GGAGGGGAGGAAGTTGCCTTGTACTGTGCCAAATATCTTCCTGATATCATCAAAGATCAGAA
GGCCTACAAGGAAGGCAAGCTACAGAAGGCTTTAGAAGATGCCTTCTTGGCTATTGACGCCA
AATTGACCACTGAAGAAGTCATTAAAGAGCTGGCACAGATTGCAGGGCGACCCACTGAGGAT
GAAGATGAAAAAGAAAAAGTAGCTGATGAAGATGATGTGGACAATGAGGAGGCTGCACTGCT
GCATGAAGAGGCTACCATGACTATTGAAGAGCTGCTGACACGCTACGGGCAGAACTGTCACA
AGGGCCCTCCCCACAGCAAATCTGGAGGTGGGACAGGCGAGGAACCAGGGTCCCAGGGCCTC
AATGGGGAGGCAGGACCTGAGGACTCAACTAGGGAAACTCCTTCACAAGAAAATGGCCCCAC
AGCCAAGGCCTACACAGGCTTTTCCTCCAACTCGGAACGTGGGACTGAGGCAGGCCAAGTTG
GTGAGCCTGGCATTCCCACTGGTGAGGCTGGGCCTTCCTGCTCTTCAGCCTCTGACAAGCTG
CCTCGAGTTGCTAAGTCCAAGTTCTTTGAGGACAGTGAGGATGAGTCAGATGAGGCGGAGGA
AGAAGAGGAAGACAGTGAGGAATGCAGCGAGGAAGAGGATGGCTACAGCAGTGAGGAGGCAG
AGAATGAGGAAGATGAGGATGACACCGAGGAGGCTGAAGAGGACGATGAAGAAGAAGAAGAA
GAGATGATGGTGCCAGGGATGGAAGGCAAAGAGGAGCCTGGCTCTGACAGTGGTACAACAGC
GGTGGTGGCCCTGATACGAGGGAAGCAGTTGATTGTAGCCAACGCAGGAGACTCTCGCTGTG
TGGTATCTGAGGCTGGCAAAGCTTTAGACATGTCCTATGATCACAAACCAGAGGATGAAGTA
GAACTAGCACGCATCAAGAATGCTGGTGGCAAGGTCACCATGGATGGGCGAGTCAACGGGGG
CCTCAACCTCTCCAGAGCCATTGGGGACCACTTCTATAAGAGAAACAAGAACCTGCCACCTG
AGGAACAGATGATTTCAGCCCTTCCTGACATCAAGGTGCTGACTCTCACTGACGACCATGAA
TTCATGGTCATTGCCTGTGATGGCATCTGGAATGTGATGAGCAGCCAGGAAGTTGTAGATTT
CATTCAATCAAAGATCAGCCAGCGTGATGAAAATGGGGAGCTTCGGTTATTGTCATCCATTG
TGGAAGAGCTGCTGGATCAGTGCCTGGCACCAGACACTTCTGGGGATGGTACAGGGTGTGAC
AACATGACCTGCATCATCATTTGCTTCAAGCCCCGAAACACAGCAGAGCTCCAGCCAGAGAG
TGGCAAGCGAAAACTAGAGGAGGTGCTCTCTACTGAGGGGGCTGAAGAAAATGGCAACAGCG
ACAAGAAGAAGAAGGCCAAGCGAGACTAG

SEQ ID NO. 4

MGAYLSQPNT VKCSGDGVGA PRLPLPYGFS AMQGWRVSME DAHNCIPELD SETAMFSVYD
GHGGEEVALY CAKYLPDIIK DQKAYKEGKL QKALEDAFLA IDAKLTTEEV IKELAQIAGR
PTEDEDEKEK VADEDDVDNE EAALLHEEAT MTIEELLTRY GQNCHKGPPH SKSGGGTGEE
PGSQGLNGEA GPEDSTRETP SQENGPTAKA YTGFSSNSER GTEAGQVGEP GIPTGEAGPS
CSSASDKLPR VAKSKFFEDS EDESDEAEEE EEDSEECSEE EDGYSSEEAE NEEDEDDTEE
AEEDDEEEEE EMMVPGMEGK EEPGSDSGTT AVVALIRGKQ LIVANAGDSR CVVSEAGKAL
DMSYDHKPED EVELARIKNA GGKVTMDGRV NGGLNLSRAI GDHFYKRNKN LPPEEQMISA
LPDIKVLTLT DDHEFMVIAC DGIWNVMSSQ EVVDFIQSKI SQRDENGELR LLSSIVEELL
DQCLAPDTSG DGTGCDNMTC IIICFKPRNT AELQPESGKR KLEEVLSTEG AEENGNSDKK
KKAKRD

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Biopharm GmbH
(B) STREET: Czernyring 22
(C) CITY: Heidelberg
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 69115

(ii) TITLE OF INVENTION: Nucleic acid encoding a novel human protein phosphatase

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 98107346.3

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 678 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TACGGGCAGA ACTGTCACAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG GACAGGCGAG 60

GAACCAGGGT CCCAGGGCCT CAATGGGGAG GCAGGACCTG AGGACTCAAC TAGGGAAACT 120

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CCTTCACAAG AAAATGGCCC CACAGCCAAG GCCTACACAG GCTTTTCCTC CAACTCGGAA     180

CGTGGGACTG AAGCAGGCCA AGTTGGTGAG CCTGGCATTC CCACTGGTGA GGCTGGGCCT     240

TCCTGCTCTT CAGCCTCTGA CAAGCTGCCT CGAGTTGCTA AGTCCAAGTT CTTTGAGGAC     300

AGTGAGGATG AGTCAGATGA GGCGGAGGAA GAAGAGGAAG ACAGTGAGGA ATGCAGCGAG     360

GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG AAGATGAGGA TGACACCGAG     420

GAGGCTGAAG AGGACGATGA AGAAGAAGAA GAAGAGATGA TGGTGCCAGG GATGGAAGGC     480

AAAGAGGAGC CTGGCTCTGA CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG     540

CAGTTGATTG TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAGCT     600

TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCACG CATCAAGAAT     660

GCTGGTGGCA AGGTCACC                                                   678
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 226 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Tyr Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly
1               5                  10                  15

Gly Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly
            20                  25                  30

Pro Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr
        35                  40                  45

Ala Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu
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                50                  55                  60

Ala Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro
65                  70                  75                  80

Ser Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys
                85                  90                  95

Phe Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu Glu
            100                 105                 110

Glu Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu
        115                 120                 125

Glu Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu
    130                 135                 140

Asp Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly
145                 150                 155                 160

Lys Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu
                165                 170                 175

Ile Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys
            180                 185                 190

Val Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys
        195                 200                 205

Pro Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys
    210                 215                 220

Val Thr
225
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1641 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGGTGCCT ACCTCTCCCA GCCCAACACG GTGAAGTGCT CCGGGGACGG GGTCGGCGCC 60

CCGCGCCTGC CGCTGCCCTA CGGCTTCTCC GCCATGCAAG GCTGGCGCGT CTCCATGGAG 120

GATGCTCACA ACTGTATTCC TGAGCTGGAC AGTGAGACAG CCATGTTTTC TGTCTACGAT 180

GGACATGGAG GGGAGGAAGT TGCCTTGTAC TGTGCCAAAT ATCTTCCTGA TATCATCAAA 240

GATCAGAAGG CCTACAAGGA AGGCAAGCTA CAGAAGGCTT TAGAAGATGC CTTCTTGGCT 300

ATTGACGCCA AATTGACCAC TGAAGAAGTC ATTAAAGAGC TGGCACAGAT TGCAGGGCGA 360

CCCACTGAGG ATGAAGATGA AAAAGAAAAA GTAGCTGATG AAGATGATGT GGACAATGAG 420

GAGGCTGCAC TGCTGCATGA AGAGGCTACC ATGACTATTG AAGAGCTGCT GACACGCTAC 480

GGGCAGAACT GTCACAAGGG CCCTCCCCAC AGCAAATCTG GAGGTGGGAC AGGCGAGGAA 540

CCAGGGTCCC AGGGCCTCAA TGGGGAGGCA GGACCTGAGG ACTCAACTAG GGAAACTCCT 600

TCACAAGAAA ATGGCCCCAC AGCCAAGGCC TACACAGGCT TTTCCTCCAA CTCGGAACGT 660

GGGACTGAGG CAGGCCAAGT TGGTGAGCCT GGCATTCCCA CTGGTGAGGC TGGGCCTTCC 720

TGCTCTTCAG CCTCTGACAA GCTGCCTCGA GTTGCTAAGT CCAAGTTCTT TGAGGACAGT 780

GAGGATGAGT CAGATGAGGC GGAGGAAGAA GAGGAAGACA GTGAGGAATG CAGCGAGGAA 840

GAGGATGGCT ACAGCAGTGA GGAGGCAGAG AATGAGGAAG ATGAGGATGA CACCGAGGAG 900

GCTGAAGAGG ACGATGAAGA AGAAGAAGAA GAGATGATGG TGCCAGGGAT GGAAGGCAAA 960

GAGGAGCCTG GCTCTGACAG TGGTACAACA GCGGTGGTGG CCCTGATACG AGGGAAGCAG 1020

TTGATTGTAG CCAACGCAGG AGACTCTCGC TGTGTGGTAT CTGAGGCTGG CAAAGCTTTA 1080

GACATGTCCT ATGATCACAA ACCAGAGGAT GAAGTAGAAC TAGCACGCAT CAAGAATGCT 1140

GGTGGCAAGG TCACCATGGA TGGGCGAGTC AACGGGGGCC TCAACCTCTC CAGAGCCATT 1200

GGGGACCACT TCTATAAGAG AAACAAGAAC CTGCCACCTG AGGAACAGAT GATTTCAGCC 1260

CTTCCTGACA TCAAGGTGCT GACTCTCACT GACGACCATG AATTCATGGT CATTGCCTGT 1320

GATGGCATCT GGAATGTGAT GAGCAGCCAG GAAGTTGTAG ATTTCATTCA ATCAAAGATC 1380

AGCCAGCGTG ATGAAAATGG GGAGCTTCGG TTATTGTCAT CCATTGTGGA AGAGCTGCTG 1440

GATCAGTGCC TGGCACCAGA CACTTCTGGG GATGGTACAG GGTGTGACAA CATGACCTGC 1500

ATCATCATTT GCTTCAAGCC CCGAAACACA GCAGAGCTCC AGCCAGAGAG TGGCAAGCGA 1560

AAACTAGAGG AGGTGCTCTC TACTGAGGGG GCTGAAGAAA ATGGCAACAG CGACAAGAAG 1620

AAGAAGGCCA AGCGAGACTA G 1641

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Ala Tyr Leu Ser Gln Pro Asn Thr Val Lys Cys Ser Gly Asp
1 5 10 15

Gly Val Gly Ala Pro Arg Leu Pro Leu Pro Tyr Gly Phe Ser Ala Met
20 25 30

Gln Gly Trp Arg Val Ser Met Glu Asp Ala His Asn Cys Ile Pro Glu
35 40 45

Leu Asp Ser Glu Thr Ala Met Phe Ser Val Tyr Asp Gly His Gly Gly
50 55 60

Glu Glu Val Ala Leu Tyr Cys Ala Lys Tyr Leu Pro Asp Ile Ile Lys
65 70 75 80

Asp Gln Lys Ala Tyr Lys Glu Gly Lys Leu Gln Lys Ala Leu Glu Asp
85 90 95

Ala Phe Leu Ala Ile Asp Ala Lys Leu Thr Thr Glu Glu Val Ile Lys
100 105 110

Glu Leu Ala Gln Ile Ala Gly Arg Pro Thr Glu Asp Glu Asp Glu Lys
115 120 125

Glu Lys Val Ala Asp Glu Asp Asp Val Asp Asn Glu Glu Ala Ala Leu
130 135 140

Leu His Glu Glu Ala Thr Met Thr Ile Glu Glu Leu Leu Thr Arg Tyr
145 150 155 160

Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly Gly
165 170 175

Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly Pro
180 185 190

Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr Ala
195 200 205

Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu Ala
210 215 220

Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro Ser
225 230 235 240

Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys Phe
245 250 255

Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu Glu Glu
260 265 270

Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu Glu
275 280 285

Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu Asp
290 295 300

Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly Lys
305 310 315 320

Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu Ile
325 330 335

Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys Val
340 345 350

Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys Pro
355 360 365

Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys Val
370 375 380

```
Thr Met Asp Gly Arg Val Asn Gly Gly Leu Asn Leu Ser Arg Ala Ile
385                 390                 395                 400

Gly Asp His Phe Tyr Lys Arg Asn Lys Asn Leu Pro Pro Glu Glu Gln
                405                 410                 415

Met Ile Ser Ala Leu Pro Asp Ile Lys Val Leu Thr Leu Thr Asp Asp
            420                 425                 430

His Glu Phe Met Val Ile Ala Cys Asp Gly Ile Trp Asn Val Met Ser
        435                 440                 445

Ser Gln Glu Val Val Asp Phe Ile Gln Ser Lys Ile Ser Gln Arg Asp
    450                 455                 460

Glu Asn Gly Glu Leu Arg Leu Leu Ser Ser Ile Val Glu Glu Leu Leu
465                 470                 475                 480

Asp Gln Cys Leu Ala Pro Asp Thr Ser Gly Asp Gly Thr Gly Cys Asp
                485                 490                 495

Asn Met Thr Cys Ile Ile Ile Cys Phe Lys Pro Arg Asn Thr Ala Glu
            500                 505                 510

Leu Gln Pro Glu Ser Gly Lys Arg Lys Leu Glu Glu Val Leu Ser Thr
        515                 520                 525

Glu Gly Ala Glu Glu Asn Gly Asn Ser Asp Lys Lys Lys Lys Ala Lys
    530                 535                 540

Arg Asp
545
```

Claims

1. A nucleic acid comprising a nucleotide sequence encoding a human serine/threonine phosphatase or a functional fragment thereof that is capable of dephosphorylating serine or threonine residues, wherein the nucleotide sequence comprises:

   (a) the nucleotides as shown in SEQ ID NO. 1 or SEQ ID NO. 3; or

   (b) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequence of (a); or

   (c) an allelic derivative of the sequences of (a) or (b); or

   (d) a DNA sequence which is capable of hybridizing to the sequences in (a), (b) and (c), and encoding a protein containing the amino acid sequence as depicted in SEQ ID NO. 2 or SEQ ID NO. 4; or

   (e) a nucleotide sequence which is capable of hybridizing to the DNA sequences in (a), (b), (c) and (d), and encoding a protein having essentially the same biological properties as the protein defined in (d).

2. The nucleic acid according to claim 1, wherein the nucleotide sequence is a vertebrate DNA sequence, a mammalian sequence, preferably a primate, human, porcine, or rodent, preferably a rat or rabbit, DNA sequence.

3. A recombinant molecule comprising a nucleic acid according to claim 1 or 2.

4. The recombinant molecule according to claim 3, wherein said nucleic acid sequence is functionally linked to an expression-control sequence.

5. A host containing the nucleic acid according to claim 1 or 2, or the recombinant molecule according to claim 3 or 4.

6. The host according to claim 5, which is a bacterium, a fungus, a plant cell, an animal or a human cell.

7. A process for the production of a PP2C-like protein comprising cultivating a host according to claim 5 or 6 and recovering said PP2C-like protein from the culture.

8. A PP2C-like protein or a biologically active fragment thereof encoded by a nucleic acid according to claim 1 or 2 or by a recombinant molecule according to claim 3 or 4.

9. The protein according to claim 8, comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4.

10. An agonist as a substitute for the protein of claim 8 or 9.

11. An antagonist directed to the protein of claim 8 or 9.

12. A pharmaceutical composition containing the protein or a biologically active fragment thereof according to claim 8 and 9 or the agonist according to claim 10 or the antagonist according to claim 11, and optionally, a pharmaceutically acceptable carrier and/or diluent.

13. The pharmaceutical composition according to claim 12 for the treatment of leukemia, brain cancer, breast cancer, prostate cancer, Alzheimer's disease, Huntingdon's disease, Parkinson's disease, and epilepsy, and of disorders of the reproductive system, or for the regulation of spermatogenesis or the maturation of mammalian germ cells.

14. An antibody or antibody fragment which is capable of specifically binding to the protein of claim 8 and 9 or to the agonist of claim 10 or to the antagonist of claim 11.

15. The antibody according to claim 14, which is a monoclonal antibody.

16. Use of an antibody or antibody fragment according to claim 14 or 15 for detecting the protein or a biologically active fragment thereof as defined in claim 8 or 9.

17. A diagnostic kit containing the agonist according to claim 10 or the antagonist according to claim 11 or the antibody or antibody fragment according to claim 14 or 15.

Fig. 1

```
MP19-PCR     YGQNCHKGPP HSKSGGGTGE EPGSQGLNGE AGPEDSTRET PSQENGPTAK   50
PP2C-Human   MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
PP2C-Rabbit  MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
PP2C-Rat     MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
                *    *          * *       **          *

MP19-PCR     AYTGFSSNSE RGTEAGQVGE PGIPTGEAGP SCSSASDKLP RVAKSKFFED  100
PP2C-Human   LPSGLESWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
PP2C-Rabbit  LPSGLETWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
PP2C-Rat     LPSGLETWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
                   *       *           *   *                    *          *              *

MP19-PCR     SEDESDEAEE EEEDSEECSE EEDGYSSEEA ENEEDEDDTE EAEEDDEEEE  150
PP2C-Human   SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID   109
PP2C-Rabbit  SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID   109
PP2C-Rat     SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID   109
             *          *                          *                  *

MP19-PCR     EEMMVPGMEG KEEPGSDSGT TAVVALIRGK QLIVANAGDS RCVVSEAGKA  200
PP2C-Human   EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV   157
PP2C-Rabbit  EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV   157
PP2C-Rat     EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV   157
             * * *   *     *          **   ***   **              * *** *          *

MP19-PCR     LDMSYDHKPE DEVELARIKN AGGKVT                            226
PP2C-Human   HFFTQDHKPS NPLEKERIQN AGGSVM                            183
PP2C-Rabbit  HFFTQDHKPS NPLEKERIQN AGGSVM                            183
PP2C-Rat     HFFTQDHKPS NPLEKERIQN AGGSVM                            183
                  ****     *  ** * *** *
```

Figure 2

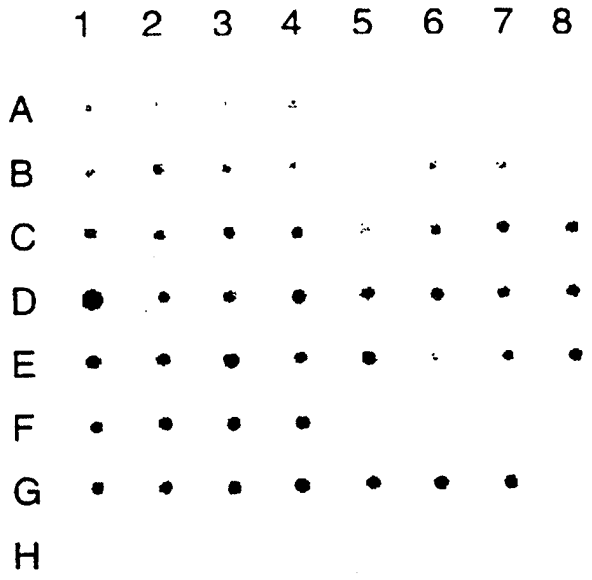
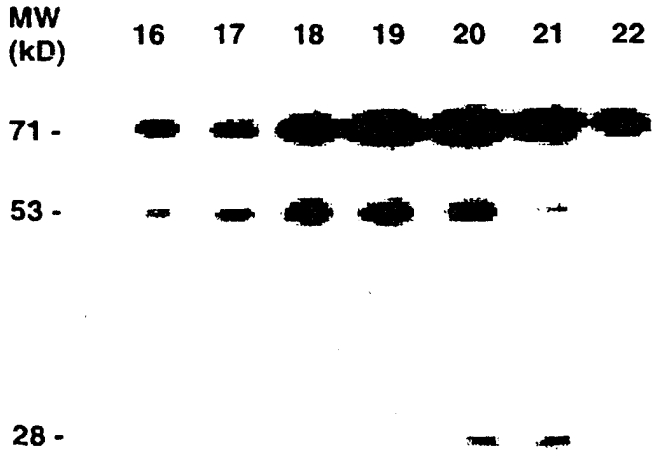


Figure 3



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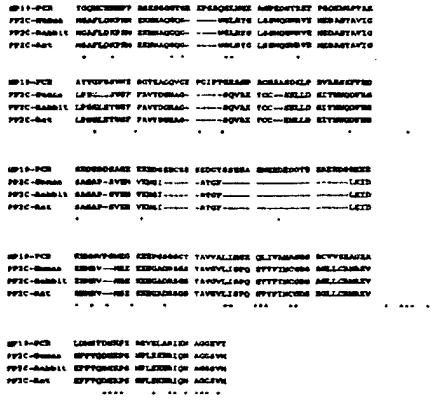
(54) Nucleic acid encoding a human protein phosphatase

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Fig. 1



EP 0 874 052 A2 CORRIGENDUM issued on 10.03.1999 (bibliography updates included) - See p.8-16

Description

The present invention relates to nucleic acids encoding a novel human protein phosphatase of the protein serine/threonine phosphatase family. In particular, it relates to novel DNA sequences encoding a serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Protein phosphorylation-dephosphorylation is a universal mechanism by which different cellular events are regulated. The serine/threonine-specific phosphatases have been classified into four main types according to their in vitro specificity for selected substrates and sensitivity to activators and inhibitors (Ingebritsen, T.S. and Cohen, P. (1983) Eur. J. Biochem. 182, 255-261). Sequence analysis revealed that they can be classified into two major gene families. The first one includes type 1 (PP1), type 2A (PP2A), and type 2B (PP2B) phosphatases, which share 37 to 59 % sequence identity (Barton, G.J. et al., (1994) Eur. J. Biochem. 220, 225-237) in their catalytic domains and are inhibited by okadaic acid (Bialojan, C., and Takai, A. (1988) Biochem. J. 256, 283-290). The second family, the Mg^{2+}-dependent phosphatases, also designated type 2C (PP2C), share little sequence similarity with the first family and are insensitive to okadaic acid. cDNA sequences of PP2C α and β from mammalian sources showed > 90 % identity. PP2Cs have been implicated in the regulation of fatty acid and cholesterol biosynthesis (Moore, F. et al. (1991) Eur. J. Biochem. 199 691-697) and heat shock response (Maeda et al. (1993) Mol. Cell. Biol. 113, 5408-5417, Shiozaki, K. et al. (1994) Mol. Cell. Biol. 14, 3742-375).

The technical problem underlying the present invention is to provide a new human PP2C-like protein phosphatase which is distinct from the other PP2Cs.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims. Other features and advantages of the invention will be apparent from the description of the preferred embodiments and drawings.

The sequence listings and drawings will now be briefly described.

SEQ ID NO. 1 shows the nucleotide sequence of MP-19, a DNA sequence derived from human placenta.

SEQ ID NO. 2 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 1.

SEQ ID NO. 3 shows the nucleotid sequence of MP-19 full-lenght cDNA derived from human placenta.

SEQ ID NO. 4 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 3.

The figures show:

Figure 1 shows the alignment of the amino acid sequence of MP-19 with some related proteins of the PP2C family. The asterisk (*) indicates the position of identical amino acids of the compared amino acid sequences.

MP19-PCR:	amino acid sequence of MP-19 (SEQ ID NO. 2)
PP2C-Human:	human protein phosphatase 2C alpha (Accession No. S87759)
PP2C-Rabbit:	rabbit protein phosphatase 2C alpha (Accession No. S87757)
PP2C-Rat:	rat protein phosphatase 2C (Accession No. J04503)

Figure 2 is a human RNA master blot. The RNA-dot blot analysis shows hybridization of MP-19 PCR probe with different human RNA samples.
A1 whole brain, A2 amygdala, A3 caudate nucleus, A4 cerebellum, A5 cerebral cortex, A6 frontal lobe, A7 hippocampus, A8 medulla oblongata, B1 occipital lobe, B2 putamen, B3 substantia nigra, B4 temporal lobe, B5 thalamus, B6 subthalamic nucleus, B7 spinal cord, B8 blank, C1 heart, C2 aorta, C3 skeletal muscle, C4 colon, C5 bladder, C6 uterus, C7 prostate, C8 stomach, D1 tests, D2 ovary, D3 pancreas, D4 pituitary gland, D5 adrenal gland, D6 thyroid gland, D7 salivary gland, D8 mammary gland, E1 kidney, E2 liver, E3 small intestine, E4 spleen, E5 thymus, E6 peripheral leukocyte, E7 lymph node, E8 bone marrow, F1 appendix, F2 lung, F3 trachea, F4 placenta, F5-F8 blank, G1 fetal brain, G2 fetal heart, G3 fetal kidney, G4 fetal liver, G5 fetal spleen, G6 fetal thymus, G7 fetal lung, G8 blank, H1 yeast total RNA, H2 yeast tRNA, H3 *E. coli* rRNA, H4 *E. coli* DNA, H5 poly r(A), H6-H8 human DNA.

Figure 3 shows the detection of MP-19 after IMAC. The Western analysis was performed after purification of MP-19 using Immobilized Metal Ion Affinity Chromatography (IMAC). Positive signals were obtained from fraction 16 to fraction 22.

The amino acid sequence alignment of MP-19 with sequences of different PP2Cs (shown in Figure 1) demonstrates the homology of MP-19 to the PP2C family but implicate also that MP-19 belongs to a new protein phosphatase group. The homology of the derived MP-19 amino acid sequence (aa 1 - aa 226) to PP2C from human, rabbit and rat displays a sequence homology of 21.2 %. Moreover a partial sequence of MP-19 (amino acid sequence 158 - 226) which is in the shown alignment not disrupted by gaps indicates a sequence homology of 39.1 % to the compared PP2Cs.

The present invention relates particularly to a novel serine/threonine protein phosphatase and, preferably, provides DNA sequences contained in the corresponding gene.

Such sequences include nucleotide sequences as illustrated in SEQ ID NO. 1 and SEQ ID NO. 3, allelic derivatives of said sequences and DNA sequences degenerated as a result of the genetic code for said sequences. It also includes DNA sequences hybridizing under stringent conditions with the DNA sequence mentioned above. It further includes antisense nucleic acid, preferably antisense MP-19 nucleic acid, directed to the above defined nucleic acid. The terms "nucleic acid sequence" and "nucleotide sequence" refers to DNA or RNA or heterooligomeric sequences, which may be double- or single-stranded.

Although said allelic, degenerate and hybridizing sequences may have structural divergences due to naturally occurring mutations, such as small deletions or substitutions, they will usually still exhibit essentially the same useful properties, allowing their use in basically the same medical or diagnostic applications.

According to the present invention, the term "hybridization" means conventional hybridization conditions, preferably conditions with a salt concentration of 6 x SSC at 62°C to 66°C followed by a one-hour wash with 0.6 x SSC, 0.1% SDS at 62°C to 66°C.

Important biological embodiments of the present invention are DNA sequences of the above and obtainable from vertebrates, preferably mammals such as pig and from rodents such as rat, and in particular from primates such as humans.

Particularly preferred embodiments of the present invention are the DNA sequence termed MP-19 which are shown in SEQ ID NO. 1 and SEQ ID No. 3. The corresponding transcripts of MP-19 were obtained from human placenta tissue and code for a protein showing considerable amino acid homology to the PP2C proteins (shown in Figure 1 deduced from SEQ ID NO. 1). The protein sequence of rabbit and human PP2C α and rat and rabbit PP2C α are described in Mann et al. (1992) Biochim. Biophys. Acta 1130, 100-104. Some typical sequence homologies, which are specific for the known PP2Cs, were also found in the MP-19 sequence. In the present invention, cloning was carried out according to the method described below. Once the DNA sequence has been cloned, the preparation of host cells capable of producing the PP2C-like protein MP-19 and the production of said protein can be easily accomplished using known recombinant DNA techniques comprising constructing the expression plasmids encoding said protein and transforming a host cell with said expression plasmid, cultivating the transformant in a suitable culture medium, and recovering the product having PP2C-like activity.

Thus, the invention also relates to recombinant molecules comprising DNA sequences as described above, optionally linked to an expression control sequence. Such vectors may be useful in the production of the PP2C-like protein in stable or transiently transformed cells. Several animal, plant, fungal and bacterial systems may be employed for the transformation and subsequent cultivation process. Preferably, expression vectors which can be used in the invention contain sequences necessary for the replication in the host cell and are autonomously replicable. It is also preferable to use vectors containing selectable marker genes which can be easily selected for transformed cells. The necessary operation is well-known to those skilled in the art.

It is another object of the invention to provide a host cell transformed by an expression plasmid of the invention and capable of producing a protein of the serine/threonine phosphatase family. Examples of suitable host cells include various eucaryotic and procaryotic cells, such as *E. coli*, insect cells, plant cells, mammalian cells, and fungi such as yeast.

Another object of the present invention is to provide a PP2C-like protein or a biologically active fragment thereof encoded by the sequences described above and displaying biological features such as dependency of Mg^{2+} (or Mn^{2+}) for activity. Furthermore, the phosphatase catalyzes dephosphorylation of phosphoseryl/threonyl residues of proteins and peptides phosphorylated by cAMP-dependent protein kinases and protein kinase C. It is insensitive to inhibitors like okadaic acid and calyculin A, heparin and PP1 inhibitors 1 and 2. It does not attack phosphorylase α. It is inhibited by polycations and F^- ions. A preferred substrate for the PP2C-like protein is the SET protein, suggesting capacities possible relevant to therapeutically treatment of leukemia. Furthermore, the PP2C-like protein prefers basic substrates such as histones, and MBP phosphorylated by cAMP-dependent protein kinase, suggesting a special function for this phosphatase in the brain. The amino acid sequences of especially preferred PP2C-like proteins (MP-19) are shown in SEQ ID NO. 2 and SEQ ID NO. 4.

It is a further aspect of the invention to provide a process for the production of PP2C-like proteins. Such a process comprises cultivating a host cell being transformed with a nucleic acid sequence of the present invention in a suitable culture medium and purifying the PP2C-like protein produced. Thus, this process allows the production of the sufficient amount of the desired protein for use in medical treatments. The host cell is obtainable from bacteria such as *Bacillus spec.* or *Escherichia coli*, from fungi such as yeast, from plants such as tobacco, potato, or *Arabidopsis*, and from animals, in particular vertebrate cell lines such as the Mo-, COS- or CHO cell line.

It is another object of the present invention to provide pharmaceutical compositions containing a therapeutically-effective amount of a PP2C-like protein of the present invention and, optionally, a pharmaceutically acceptable carrier and/or diluent, and/or agonists and/or antagonists thereof. Such a therapeutic composition can be used for the treatment of cancer such as leukemia, brain cancer, breast cancer and prostate cancer. The pharmaceutical composition according to the present invention can also be therapeutically applied for degenerative disorders of the CNS, e.g. Alzheimer's disease, Huntingdon's disease, Parkinson's disease, and epilepsy, and disorders of the reproductive system e.g. fertility disorders or testicular cancer. Another possible clinical application of a PP2C-like protein is the use for treatment of liver diseases, diabetes, and cystic fibrosis. The pharmaceutical composition comprising the protein of the present invention can also be used for treatment of microbial or viral infections. Another application of the pharmaceutical composition of the present invention is the usage of the protein in the regulation of spermatogenesis or the maturation of mammalian germ cells, e.g. for centraception.

Furthermore, the application of the composition is not limited to humans but can include animals, in particular domestic animals, as well.

Finally, another object of the present invention is an antibody or antibody fragment, which is capable of specifically binding to the proteins of the present invention. Methods to raise such specific antibody are known in the art. Such an antibody is preferably a monoclonal antibody. Such antibodies or antibody fragments might be useful for diagnostic methods.

The following examples illustrate in more detail the present invention, but should not be construed as limiting the invention.

Example 1

Isolation of MP-19

For the reverse transcription reaction, 5 µg total RNA (0.5 µg/µl) derived from human placenta tissue was heated for 5 minutes and cooled rapidly on ice for 5 minutes. The reverse transcription reagent mixture containing 5 µg total RNA, 38 u of RNA-guard (Pharmacia), 2.5 µg oligomer d(T)12-18 (Boehringer Mannheim), 5x reaction buffer (250 mM Tris/HCl pH 8.5; 50 mM $MgCl_2$; 50 mM DTT; 600 mM KCl), 10 mM of each dNTP (Pharmacia), 37.5 u of avian myoblastosis virus reverse transcriptase (AMV, Boehringer Mannheim). The reaction mixture (20 µl) was incubated for 90 minutes at 42°C. The resulting placenta cDNA pool was stored at -20°C.

For the primary polymerase chain reaction (PCR), a placenta-derived cDNA pool was used as template in a 50 µl reaction mixture. The PCR reaction was carried out in a RoboCycler Gradient 96 (Stratagene). The amplification was performed in 1x PCR-buffer (10 mM Tris/HCl pH 8.3; 50 mM KCl; 0.001 % gelatine), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide (ALK6-N2, 5' - TT(CT)(AG)C(AGCT)AT(AGCT)ATAGAAGAAGATGA - 3' and ALK6-R2, 5' - CC(AGCT)CGCCA(CT)TT(AGCT)CCCATCCA - 3') and 1.5 u Taq polymerase (Perkin Elmer). The PCR reaction contained cDNA corresponding to 30 ng of total RNA as starting material. The reaction mixture was overlaid by 40 µl paraffin incubated for 180s/94°C and subjected to 30 cycles (50s/94°C, 90s/48 °C, 60s/72 °C) with an additional extension for 480s/72 °C in the Thermocycler.

A second round of amplification was performed as described above with exception that 5 µl from the first PCR reaction was used as template DNA for the PCR. A 10 µl sample from the second PCR amplification was fractionated by electrophoresis using a 2 % agarose gel in TBE buffer. After electrophoresis amplified DNA corresponding to a molecular weight of about 600-800 bp was excised from the gel and isolated by 3x freeze/thaw cycles (-20 °C/ + 37 °C) and using the DNA Purification Kit "Easy Pure" (Biozyme, Cat. no. 39001) following the instructions of the manufacturer.

The eluted DNA was amplified a third time as described in the primary PCR with exception that 3 µl of the eluted DNA, resulted from the second round of amplification, was used as template for PCR and the annealing temperature was 56 °C instead of 48 °C. After electrophoresis using a 2% agarose gel in TBE buffer, a distinct DNA band that corresponds to a molecular weight of about 700 bp was eluted with the extraction method described before. After than an additionally purification using the QIAquick 8 PCR Purification Kit (Qiagen, Cat. no. 28144) following the instructions of the manufacturer, was carried out.

Cloning of the purified DNA was established using the Original TA Cloning Kit (Invitrogen, Cat. no. K2000-40). Plasmid DNA from positive clones was isolated with the QIAwell 8 Plus Plasmid Kit (Qiagen, Cat. no. 16142) and sequenced with an automatic DNA sequencer (ALFexpress, Pharmacia). The resulting DNA sequence was analyzed by a homol-

ogy search with the blast program.

Example 2

Isolation of MP-19 full-length cDNA

Isolation of a full-length cDNA clone of MP-19 was performed with a commercial available Human Plac Lambda cDNA Library (Stratagene, Cat. no. 937225). For screening, a labeled PCR probe was generated from M DNA (SEQ ID NO. 1). The amplification was performed in 1x PCR-buffer (Qiagen, Germany), 1 mM of dATP, 1 m dCTP, 1 mM of dGTP, 0.6 mM of dTTP (Pharmacia, Germany), 0.4 mM of Digoxigenin-11-dUTP (Boehringer, nheim), 100 pmol of each oligonucleotide PL19-N1 (5'-GGGCAGAACTGTCACAAGGG-3') and PL19-R1 (5'-CATC GGTGACCTTGCCACC-3') and 1 u Taq DNA-polymerase (Qiagen). The PCR mix was overlaid by 40 µl par incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/58 °C, 60s/72 °C) with an additional extensic 180s/72 °C.

Prehybridization of plaque lift filters from cDNA library were done at 58 °C for 4 h in 0.25 M Na_2HPO_4, 7 % SI % BSA, 1 mM EDTA, pH 7.2. Hybridization was carried out with 50 ng labeled MP19 PCR probe for 15 h under s buffer conditions as prehybridization was done. Filter washed 3 times (5 min, 10 min and 15 min) with 30 mM Na_2H 0,1 % SDS at 60 °C. Detection of signals were performed with DIG Luminescent detection kit from Boehringer, I nheim (Cat. no. 1363514). Proceed from a positive signal, clone 39-1 was isolated and sequenced. The resulting D sequence from clone 39-1 conform to Bp. 387-1641 in SEQ ID NO. 3. To generate the 5'cDNA end of MP-19 full-le cDNA, 1 µg of a Lambda-DNA preparation from Human Placenta Lambda cDNA Library was subjected to PCR. ampilfication was performed in 1x PCR-buffer (Qiagen), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligon otide MP19-E5 (5'-GGATCCATGGGTGCCTACCTCTCCCAGCCC-3') which was derived from an EST sequ (accession no. AA115688) and MP19-3 (5'-GCCTGTGTAGGCCTTGGCTGTGGGGCC-3') and 1 u Taq DNA-poly ase (Qiagen). The reaction was overlaid by 40 µl paraffin incubated for 180s/94 °C and subjected to 30 cycles (60 °C, 60s/74 °C, 60s/72 °C) with an additional extension for 300s/72 °C. The resulting PCR fragment was subclone vector pCR 2.1 (Clontech, Germany). Corresponding DNA was digested with restriction endonucleases Bam HI Stu I. After that MP-19 Bam HI/Stu I fragment was inserted into clone 39-1. The resulted clone was named 28-9 w DNA-sequence present the full-lenght cDNA-sequence of MP-19 as shown in SEQ ID NO. 3. The corresponding ar acid sequence of MP-19 is shown in SEQ ID NO. 4.

Gene expression of MP-19 in human tissues

Relative expression of the MP-19 gene was determined by Northern blot analyses. A commercial available Hu RNA Master Blot (Clontech, Germany, Cat. no. 7770-1, Lot no. 7090716) was hybridized with the digoxigenin lab MP-19 PCR probe as described in: Isolation of MP-19 full-length cDNA. 50 different human tissues samples were in tigated for MP-19 gene expression. Additionally 8 different negative controls from *E. coli*, yeast and human genc DNA were applied.

Main expression of MP-19 was detected in human testis which is shown in figure 2. Lower expression of MP-19 detected in human pituitary gland, thymus, small intestine and fetal liver. Basal expression of MP-19 was found i other human RNA samples. No hybridization signals were detected in negative controls.

Expression of MP-19 cDNA in *E. coli*

The cDNA of clone 28-9 was subcloned into the expression plasmid pQE-16 (Qiagen, Germany). This cloning s egy constituted an additional tag of 6 histidine residues at the C-terminus of MP-19. pQE-16 was digested with Bar and Bgl II. The 5' part of MP-19 was excised from clone 28-9 with Bam HI and Sac I. To constitute a compatible 3' of MP-19 for cloning into plasmid pQE-16, a PCR was performed with primer MP19N-Sac I (5'-ACAGCAGAGC CAGCCAGAG-3') and MP19R-Bgl II (5'-AGATCTGTCTCGCTTGGCCTTCTTCTTC-3') and template DNA of clone 9.5' and 3'end of MP-19 DNA was ligated into pQE-16 to establish MP-19 with His-tag, which was expressed in *E.* strain M15 (Qiagen). For recombinant expression of MP-19, cells were grown in a 5 l fermenter (Bio Console ADI 1(Applikon, Netherlands) at 37 °C in LB-Medium until an OD_{600} of 2,5 was reached. After induction with 1 mM β-D ogalactopyranoside, cells were grown for additional 4 h until OD_{600} of 9,7 was reached. Cells were harvested by c trifugation at 10.000 x g for 30 min, washed once in 500 ml 1 x PBS buffer (30 min at 10.000 x g) and were froze aliquots at -80 °C. For preparation of MP-19 protein, 10 g cells were lysed in 100 ml lysis buffer (50 mM NaH_2PO_4, 8,0, 300 mM NaCl, 10 mM imidazole, 100 mg lysozyme (Serva, Germany) and 50 u Benzonase (Merck, Germany)) lowing by sonication 3 times with a ultrasonic processor (UP-200S, Dr. Hielscher GmbH, Germany) for 3 min a $kWsec^{-1}$ in an ice/water bath. Cell debris was removed by centrifugation for 30 min at 4 °C and 25.000 x g.

Purification of recombinant MP-19

Recombinant expressed MP-19 was purified using Immobilized Metal Ion Affinity Chromatography (IMAC) and Reverse Phase Liquid Chromatography (RPLC). The chromatographic purification was realized using the ÄKTA-Explorer system (Pharmacia Biotech, Germany). For IMAC, a 1 ml Hi-Trap cheating column (Pharmacia Biotech) is used. Hi-Trap column was activated with 5 column volumes (cv) of 100 mM $NiSO_4$, afterwards the column washed with 5 cv water to remove unbound Ni^{2+}. Column equilibration was performed with 5 cv of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazole, pH 8,0). Cell lysat results from 1 g *E. coli* cells was loaded onto column. Afterwards the column washed with lysis buffer to remove unbound protein. Protein were eluted using the following gradient program. Step 1: 20 mM imidazole to 300 mM imidzole within 20 minutes, step 2: 300 mM imidazol to 500 mM imidazol within 10 minutes using buffer A (50 mM Tris, 300 mM NaCl, 20 mM Imidzole, pH 6,0) and buffer B (50 mM Tris, 300 mM NaCl, 500 mM Imidazole, pH 6,0). Flow rate of chromatography were 1 ml/min, detection were performed at 280 nm. Fractions were analyzed by immunological detection, shown in Figure 3. For further purification, positive fractions containing MP-19 were pooled and loaded onto a Resource RPC (3ml) column (Pharmacia Biotech). Column was equilibrated with buffer A (0.1 % trifluor acetic acid) and protein eluted with a linear gradient of buffer B (0,1 % TFA-90 % acetonitrile). Flow rate of chromatography were 3 ml/min, detection were performed at 215 nm.

Immunolonical Detection of MP-19

Immunological detection of recombinant MP-19 was performed by western blotting using a commercial available monoclonal mouse antibody against histidine-tag (Dianova, Germany, Cat. no. dia900) in combination with Western Light chemoluminescent detection system using the goat anti-mouse-AP antibody (Tropix, U.S.A.).

Activity assay for recombinant MP-19

A Serine/Threonine Phosphatase Assay System (Promega, Germany, Cat. no. V2460) was used to determine enzymatic activity of recombinant MP-19. This assay use a chemically synthesized phosphopeptide RRA(pT)VA which is a functional substrate for MP-19 phosphatase. The amount of free phosphate which is generated by MP-19 enzymatic reaction was measured by the absorbency of a molybdate:malachit green:phosphate complex (Ekman P. and Jager O. (1993), Anal. Biochem. 214, 138-141, Deana A. D. et al. (1990), Biochimica et Biophysica Acta 1051, 199-202). Assays were performed as described by the manufacturer in PPTase-2C buffer (50 mM imidazole, pH 7,2, 0,2 mM EGTA, 5 mM $MgCl_2$, 0.02 % β-mercaptoethanol, 0,1 mg/ml BSA). To determine background of this assay, clone pQE-16-dhfr (Qiagen, Germany) was used, which is identical to pQE-MP-19 with exception that vector pQE-16 inserted a mouse dhfr gene instead of MP-19 phosphatase gene.

Results of activity assay of MP-19 shown in table 1. MP-19 has a significant activity in a $MgCl_2$ containing buffer, but no activity in a $CaCl_2$ containing buffer, which shows the requirement to Mg^{2+}. Inhibitors like okadaic acid (10 µM) shows no significant reduction of MP-19 activity. Control expression of the mouse dhfr gene shows no activity in the Serine/Threonine Phosphatase Assay System.

	A	B	C	D (Average A-C)
1	0.000	0.006	-0.006	0.000
2	0.024	0.029	0.023	0.025
3	0.304	0.291	0.298	0.298
4	0.612	0.594	0.597	0.601
5	1.080	1.140	1.137	1.119
6	0.021	0.006	0.018	0.015
7	0.025	0.043	0.018	0.029
8	0.038	0.020	0.018	0.025
9	0.030	0.012	0.013	0.018
10	0.151	0.108	0.174	0.144
11	0.146	0.147	0.139	0.144

Table 1: Activity test of MP-19

A1-D1: Phosphate standard O pmol
A2-D2: Phosphate standard 100 pmol
A3-D3: Phosphate standard 500 pmol
A4-D4: Phosphate standard 1000 pmol
A5-D5: Phosphate standard 2000 pmol
A6-D6: mouse dhfr gene with substrate (negative control)
A7-D7: mouse dhfr gene without substrate (negative control)
A8-D8: MP-19 with modified PPTase-2C buffer (5mM $MgCl_2$ is replaced by 5 mM $CaCl_2$) and substrate
A9-D9: MP-19 with PPTase-2C buffer without substrate
A10-D10: MP-19 with PPTase-2C buffer and substrate
A11-D11: MP-19 with PPTase-2C buffer, substrate and 10 μM okadaic acid

Annex to the description

Sequence listing

SEQ ID NO. 1

```
TACGGGCAGA ACTGTCACAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG      50
GACAGGCGAG GAACCAGGGT CCCAGGGCCT CAATGGGGAG GCAGGACCTG     100
AGGACTCAAC TAGGGAAACT CCTTCACAAG AAAATGGCCC CACAGCCAAG     150
GCCTACACAG GCTTTTCCTC CAACTCGGAA CGTGGGACTG AAGCAGGCCA     200
AGTTGGTGAG CCTGGCATTC CCACTGGTGA GGCTGGGCCT TCCTGCTCTT     250
CAGCCTCTGA CAAGCTGCCT CGAGTTGCTA AGTCCAAGTT CTTTGAGGAC     300
AGTGAGGATG AGTCAGATGA GGCGGAGGAA GAAGAGGAAG ACAGTGAGGA     350
ATGCAGCGAG GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG     400
AAGATGAGGA TGACACCGAG GAGGCTGAAG AGGACGATGA AGAAGAAGAA     450
GAAGAGATGA TGGTGCCAGG GATGGAAGGC AAAGAGGAGC CTGGCTCTGA     500
CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG CAGTTGATTG     550
TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAGCT     600
TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCACG     650
CATCAAGAAT GCTGGTGGCA AGGTCACC                             678
```

SEQ ID NO. 2

```
YGQNCHKGPP HSKSGGGTGE EPGSQGLNGE AGPEDSTRET PSQENGPTAK      50
AYTGFSSNSE RGTEAGQVGE PGIPTGEAGP SCSSASDKLP RVAKSKFFED     100
SEDESDEAEE EEEDSEECSE EEDGYSSEEA ENEEDEDDTE EAEEDDEEEE     150
EEMMVPGMEG KEEPGSDSGT TAVVALIRGK QLIVANAGDS RCVVSEAGKA     200
LDMSYDHKPE DEVELARIKN AGGKVT                               226
```

SEQ ID NO. 3

ATGGGTGCCTACCTCTCCCAGCCCAACACGGTGAAGTGCTCCGGGGACGGGGTCGGCGCCCC
GCGCCTGCCGCTGCCCTACGGCTTCTCCGCCATGCAAGGCTGGCGCGTCTCCATGGAGGATG
CTCACAACTGTATTCCTGAGCTGGACAGTGAGACAGCCATGTTTTCTGTCTACGATGGACAT
GGAGGGGAGGAAGTTGCCTTGTACTGTGCCAAATATCTTCCTGATATCATCAAAGATCAGAA
GGCCTACAAGGAAGGCAAGCTACAGAAGGCTTTAGAAGATGCCTTCTTGGCTATTGACGCCA
AATTGACCACTGAAGAAGTCATTAAAGAGCTGGCACAGATTGCAGGGCGACCCACTGAGGAT
GAAGATGAAAAAGAAAAAGTAGCTGATGAAGATGATGTGGACAATGAGGAGGCTGCACTGCT
GCATGAAGAGGCTACCATGACTATTGAAGAGCTGCTGACACGCTACGGGCAGAACTGTCACA
AGGGCCCTCCCCACAGCAAATCTGGAGGTGGGACAGGCGAGGAACCAGGGTCCCAGGGCCTC
AATGGGGAGGCAGGACCTGAGGACTCAACTAGGGAAACTCCTTCACAAGAAAATGGCCCCAC
AGCCAAGGCCTACACAGGCTTTTCCTCCAACTCGGAACGTGGGACTGAGGCAGGCCAAGTTG
GTGAGCCTGGCATTCCCACTGGTGAGGCTGGGCCTTCCTGCTCTTCAGCCTCTGACAAGCTG
CCTCGAGTTGCTAAGTCCAAGTTCTTTGAGGACAGTGAGGATGAGTCAGATGAGGCGGAGGA
AGAAGAGGAAGACAGTGAGGAATGCAGCGAGGAAGAGGATGGCTACAGCAGTGAGGAGGCAG
AGAATGAGGAAGATGAGGATGACACCGAGGAGGCTGAAGAGGACGATGAAGAAGAAGAAGAA
GAGATGATGGTGCCAGGGATGGAAGGCAAAGAGGAGCCTGGCTCTGACAGTGGTACAACAGC
GGTGGTGGCCCTGATACGAGGGAAGCAGTTGATTGTAGCCAACGCAGGAGACTCTCGCTGTG
TGGTATCTGAGGCTGGCAAAGCTTTAGACATGTCCTATGATCACAAACCAGAGGATGAAGTA
GAACTAGCACGCATCAAGAATGCTGGTGGCAAGGTCACCATGGATGGGCGAGTCAACGGGGG
CCTCAACCTCTCCAGAGCCATTGGGGACCACTTCTATAAGAGAAACAAGAACCTGCCACCTG
AGGAACAGATGATTTCAGCCCTTCCTGACATCAAGGTGCTGACTCTCACTGACGACCATGAA
TTCATGGTCATTGCCTGTGATGGCATCTGGAATGTGATGAGCAGCCAGGAAGTTGTAGATTT
CATTCAATCAAAGATCAGCCAGCGTGATGAAAATGGGGAGCTTCGGTTATTGTCATCCATTG
TGGAAGAGCTGCTGGATCAGTGCCTGGCACCAGACACTTCTGGGGATGGTACAGGGTGTGAC
AACATGACCTGCATCATCATTTGCTTCAAGCCCCGAAACACAGCAGAGCTCCAGCCAGAGAG
TGGCAAGCGAAAACTAGAGGAGGTGCTCTCTACTGAGGGGGCTGAAGAAAATGGCAACAGCG
ACAAGAAGAAGAAGGCCAAGCGAGACTAG

SEQ ID NO. 4

MGAYLSQPNT VKCSGDGVGA PRLPLPYGFS AMQGWRVSME DAHNCIPELD SETAMFSVYD
GHGGEEVALY CAKYLPDIIK DQKAYKEGKL QKALEDAFLA IDAKLTTEEV IKELAQIAGR
PTEDEDEKEK VADEDDVDNE EAALLHEEAT MTIEELLTRY GQNCHKGPPH SKSGGGTGEE
PGSQGLNGEA GPEDSTRETP SQENGPTAKA YTGFSSNSER GTEAGQVGEP GIPTGEAGPS
CSSASDKLPR VAKSKFFEDS EDESDEAEEE EEDSEECSEE EDGYSSEEAE NEEDEDDTEE
AEEDDEEEEE EMMVPGMEGK EEPGSDSGTT AVVALIRGKQ LIVANAGDSR CVVSEAGKAL
DMSYDHKPED EVELARIKNA GGKVTMDGRV NGGLNLSRAI GDHFYKRNKN LPPEEQMISA
LPDIKVLTLT DDHEFMVIAC DGIWNVMSSQ EVVDFIQSKI SQRDENGELR LLSSIVEELL
DQCLAPDTSG DGTGCDNMTC IIICFKPRNT AELQPESGKR KLEEVLSTEG AEENGNSDKK
KKAKRD

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Biopharm GmbH
(B) STREET: Czernyring 22
(C) CITY: Heidelberg
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 69115

(ii) TITLE OF INVENTION: Nucleic acid encoding a novel human protein phosphatase

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 98107346.3

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 678 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TACGGGCAGA ACTGTCACAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG GACAGGCGAG 60

GAACCAGGGT CCCAGGGCCT CAATGGGGAG GCAGGACCTG AGGACTCAAC TAGGGAAACT 120

CCTTCACAAG AAAATGGCCC CACAGCCAAG GCCTACACAG GCTTTTCCTC CAACTCGGAA 180

CGTGGGACTG AAGCAGGCCA AGTTGGTGAG CCTGGCATTC CCACTGGTGA GGCTGGGCCT 240

TCCTGCTCTT CAGCCTCTGA CAAGCTGCCT CGAGTTGCTA AGTCCAAGTT CTTTGAGGAC 300

AGTGAGGATG AGTCAGATGA GGCGGAGGAA GAAGAGGAAG ACAGTGAGGA ATGCAGCGAG 360

GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG AAGATGAGGA TGACACCGAG 420

GAGGCTGAAG AGGACGATGA AGAAGAAGAA GAAGAGATGA TGGTGCCAGG GATGGAAGGC 480

AAAGAGGAGC CTGGCTCTGA CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG 540

CAGTTGATTG TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAGCT 600

TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCACG CATCAAGAAT 660

GCTGGTGGCA AGGTCACC 678

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 226 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Tyr Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly
1 5 10 15

Gly Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly
20 25 30

Pro Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr
35 40 45

Ala Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu

```
     50                  55                  60

Ala Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro
65                  70                  75                  80

Ser Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys
                85                  90                  95

Phe Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu Glu
            100                 105                 110

Glu Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu
        115                 120                 125

Glu Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu
    130                 135                 140

Asp Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly
145                 150                 155                 160

Lys Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu
                165                 170                 175

Ile Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys
            180                 185                 190

Val Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys
        195                 200                 205

Pro Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys
    210                 215                 220

Val Thr
225
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1641 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGGTGCCT ACCTCTCCCA GCCCAACACG GTGAAGTGCT CCGGGGACGG GGTCGGCGCC 60

CCGCGCCTGC CGCTGCCCTA CGGCTTCTCC GCCATGCAAG GCTGGCGCGT CTCCATGGAG 120

GATGCTCACA ACTGTATTCC TGAGCTGGAC AGTGAGACAG CCATGTTTTC TGTCTACGAT 180

GGACATGGAG GGGAGGAAGT TGCCTTGTAC TGTGCCAAAT ATCTTCCTGA TATCATCAAA 240

GATCAGAAGG CCTACAAGGA AGGCAAGCTA CAGAAGGCTT TAGAAGATGC CTTCTTGGCT 300

ATTGACGCCA AATTGACCAC TGAAGAAGTC ATTAAAGAGC TGGCACAGAT TGCAGGGCGA 360

CCCACTGAGG ATGAAGATGA AAAAGAAAAA GTAGCTGATG AAGATGATGT GGACAATGAG 420

GAGGCTGCAC TGCTGCATGA AGAGGCTACC ATGACTATTG AAGAGCTGCT GACACGCTAC 480

GGGCAGAACT GTCACAAGGG CCCTCCCCAC AGCAAATCTG GAGGTGGGAC AGGCGAGGAA 540

CCAGGGTCCC AGGGCCTCAA TGGGGAGGCA GGACCTGAGG ACTCAACTAG GGAAACTCCT 600

TCACAAGAAA ATGGCCCCAC AGCCAAGGCC TACACAGGCT TTTCCTCCAA CTCGGAACGT 660

GGGACTGAGG CAGGCCAAGT TGGTGAGCCT GGCATTCCCA CTGGTGAGGC TGGGCCTTCC 720

TGCTCTTCAG CCTCTGACAA GCTGCCTCGA GTTGCTAAGT CCAAGTTCTT TGAGGACAGT 780

GAGGATGAGT CAGATGAGGC GGAGGAAGAA GAGGAAGACA GTGAGGAATG CAGCGAGGAA 840

GAGGATGGCT ACAGCAGTGA GGAGGCAGAG AATGAGGAAG ATGAGGATGA CACCGAGGAG 900

GCTGAAGAGG ACGATGAAGA AGAAGAAGAA GAGATGATGG TGCCAGGGAT GGAAGGCAAA 960

GAGGAGCCTG GCTCTGACAG TGGTACAACA GCGGTGGTGG CCCTGATACG AGGGAAGCAG 1020

TTGATTGTAG CCAACGCAGG AGACTCTCGC TGTGTGGTAT CTGAGGCTGG CAAAGCTTTA 1080

GACATGTCCT ATGATCACAA ACCAGAGGAT GAAGTAGAAC TAGCACGCAT CAAGAATGCT 1140

GGTGGCAAGG TCACCATGGA TGGGCGAGTC AACGGGGGCC TCAACCTCTC CAGAGCCATT 1200

GGGGACCACT TCTATAAGAG AAACAAGAAC CTGCCACCTG AGGAACAGAT GATTTCAGCC 1260

CTTCCTGACA TCAAGGTGCT GACTCTCACT GACGACCATG AATTCATGGT CATTGCCTGT 1320

GATGGCATCT GGAATGTGAT GAGCAGCCAG GAAGTTGTAG ATTTCATTCA ATCAAAGATC 1380

AGCCAGCGTG ATGAAAATGG GGAGCTTCGG TTATTGTCAT CCATTGTGGA AGAGCTGCTG 1440

GATCAGTGCC TGGCACCAGA CACTTCTGGG GATGGTACAG GGTGTGACAA CATGACCTGC 1500

ATCATCATTT GCTTCAAGCC CCGAAACACA GCAGAGCTCC AGCCAGAGAG TGGCAAGCGA 1560

AAACTAGAGG AGGTGCTCTC TACTGAGGGG GCTGAAGAAA ATGGCAACAG CGACAAGAAG 1620

AAGAAGGCCA AGCGAGACTA G 1641

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Ala Tyr Leu Ser Gln Pro Asn Thr Val Lys Cys Ser Gly Asp
1 5 10 15

Gly Val Gly Ala Pro Arg Leu Pro Leu Pro Tyr Gly Phe Ser Ala Met
20 25 30

Gln Gly Trp Arg Val Ser Met Glu Asp Ala His Asn Cys Ile Pro Glu
35 40 45

Leu Asp Ser Glu Thr Ala Met Phe Ser Val Tyr Asp Gly His Gly Gly
50 55 60

Glu Glu Val Ala Leu Tyr Cys Ala Lys Tyr Leu Pro Asp Ile Ile Lys
65 70 75 80

Asp Gln Lys Ala Tyr Lys Glu Gly Lys Leu Gln Lys Ala Leu Glu Asp
85 90 95

Ala Phe Leu Ala Ile Asp Ala Lys Leu Thr Thr Glu Glu Val Ile Lys
100 105 110

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Glu Leu Ala Gln Ile Ala Gly Arg Pro Thr Glu Asp Glu Asp Glu Lys
        115                 120                 125

Glu Lys Val Ala Asp Glu Asp Asp Val Asp Asn Glu Glu Ala Ala Leu
    130                 135                 140

Leu His Glu Glu Ala Thr Met Thr Ile Glu Glu Leu Leu Thr Arg Tyr
145                 150                 155                 160

Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly Gly
                165                 170                 175

Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly Pro
            180                 185                 190

Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr Ala
        195                 200                 205

Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu Ala
    210                 215                 220

Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro Ser
225                 230                 235                 240

Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys Phe
                245                 250                 255

Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu Glu Glu
            260                 265                 270

Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu Glu
        275                 280                 285

Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu Asp
    290                 295                 300

Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly Lys
305                 310                 315                 320

Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu Ile
                325                 330                 335

Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys Val
            340                 345                 350

Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys Pro
        355                 360                 365

Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys Val
    370                 375                 380
```

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Thr Met Asp Gly Arg Val Asn Gly Gly Leu Asn Leu Ser Arg Ala Ile
385                 390                 395                 400

Gly Asp His Phe Tyr Lys Arg Asn Lys Asn Leu Pro Pro Glu Glu Gln
                405                 410                 415

Met Ile Ser Ala Leu Pro Asp Ile Lys Val Leu Thr Leu Thr Asp Asp
            420                 425                 430

His Glu Phe Met Val Ile Ala Cys Asp Gly Ile Trp Asn Val Met Ser
        435                 440                 445

Ser Gln Glu Val Val Asp Phe Ile Gln Ser Lys Ile Ser Gln Arg Asp
    450                 455                 460

Glu Asn Gly Glu Leu Arg Leu Leu Ser Ser Ile Val Glu Glu Leu Leu
465                 470                 475                 480

Asp Gln Cys Leu Ala Pro Asp Thr Ser Gly Asp Gly Thr Gly Cys Asp
                485                 490                 495

Asn Met Thr Cys Ile Ile Ile Cys Phe Lys Pro Arg Asn Thr Ala Glu
            500                 505                 510

Leu Gln Pro Glu Ser Gly Lys Arg Lys Leu Glu Glu Val Leu Ser Thr
        515                 520                 525

Glu Gly Ala Glu Glu Asn Gly Asn Ser Asp Lys Lys Lys Lys Ala Lys
    530                 535                 540

Arg Asp
545
```

Claims

1. A nucleic acid comprising a nucleotide sequence encoding a human serine/threonine phosphatase or a functional fragment thereof that is capable of dephosphorylating serine or threonine residues, wherein the nucleotide sequence comprises:

   (a) the nucleotides as shown in SEQ ID NO. 1 or SEQ ID NO. 3; or

   (b) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequence of (a); or

   (c) an allelic derivative of the sequences of (a) or (b); or

   (d) a DNA sequence which is capable of hybridizing to the sequences in (a), (b) and (c), and encoding a protein containing the amino acid sequence as depicted in SEQ ID NO. 2 or SEQ ID NO. 4; or

   (e) a nucleotide sequence which is capable of hybridizing to the DNA sequences in (a), (b), (c) and (d), and encoding a protein having essentially the same biological properties as the protein defined in (d).

2. The nucleic acid according to claim 1, wherein the nucleotide sequence is a vertebrate DNA sequence, a mammalian sequence, preferably a primate, human, porcine, or rodent, preferably a rat or rabbit, DNA sequence.

3. A recombinant molecule comprising a nucleic acid according to claim 1 or 2.

4. The recombinant molecule according to claim 3, wherein said nucleic acid sequence is functionally linked to an expression-control sequence.

5. A host containing the nucleic acid according to claim 1 or 2, or the recombinant molecule according to claim 3 or 4.

6. The host according to claim 5, which is a bacterium, a fungus, a plant cell, an animal or a human cell.

7. A process for the production of a PP2C-like protein comprising cultivating a host according to claim 5 or 6 and recovering said PP2C-like protein from the culture.

8. A PP2C-like protein or a biologically active fragment thereof encoded by a nucleic acid according to claim 1 or 2 or by a recombinant molecule according to claim 3 or 4.

9. The protein according to claim 8, comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4.

10. An agonist as a substitute for the protein of claim 8 or 9.

11. An antagonist directed to the protein of claim 8 or 9.

12. A pharmaceutical composition containing the protein or a biologically active fragment thereof according to claim 8 and 9 or the agonist according to claim 10 or the antagonist according to claim 11, and optionally, a pharmaceutically acceptable carrier and/or diluent.

13. The pharmaceutical composition according to claim 12 for the treatment of leukemia, brain cancer, breast cancer, prostate cancer, Alzheimer's disease, Huntingdon's disease, Parkinson's disease, and epilepsy, and of disorders of the reproductive system, or for the regulation of spermatogenesis or the maturation of mammalian germ cells.

14. An antibody or antibody fragment which is capable of specifically binding to the protein of claim 8 and 9 or to the agonist of claim 10 or to the antagonist of claim 11.

15. The antibody according to claim 14, which is a monoclonal antibody.

16. Use of an antibody or antibody fragment according to claim 14 or 15 for detecting the protein or a biologically active fragment thereof as defined in claim 8 or 9.

17. A diagnostic kit containing the agonist according to claim 10 or the antagonist according to claim 11 or the antibody or antibody fragment according to claim 14 or 15.

Fig. 1

```
MP19-PCR     YGQNCHKGPP HSKSGGGTGE EPGSQGLNGE AGPEDSTRET PSQENGPTAK   50
PP2C-Human   MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
PP2C-Rabbit  MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45
PP2C-Rat     MGAFLDKPKM EKHNAQGQG- ----NGLRYG LSSMQGWRVE MEDAHTAVIG   45

MP19-PCR     AYTGFSSNSE RGTEAGQVGE PGIPTGEAGP SCSSASDKLP RVAKSKFFED  100
PP2C-Human   LPSGLESWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
PP2C-Rabbit  LPSGLETWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87
PP2C-Rat     LPSGLETWSF FAVYDGHAG- -----SQVAK YCC--EHLLD HITNNQDFKG   87

MP19-PCR     SEDESDEAEE EEEDSEECSE EEDGYSSEEA ENEEDEDDTE EAEEDDEEEE  150
PP2C-Human   SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID  109
PP2C-Rabbit  SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID  109
PP2C-Rat     SAGAP-SVEN VKNGI----- -RTGF----- ---------- ------LEID  109

MP19-PCR     EEMMVPGMEG KEEPGSDSGT TAVVALIRGK QLIVANAGDS RCVVSEAGKA  200
PP2C-Human   EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV  157
PP2C-Rabbit  EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV  157
PP2C-Rat     EHMRV--MSE KKHGADRSGS TAVGVLISPQ HTYFINCGDS RGLLCRNRKV  157

MP19-PCR     LDMSYDHKPE DEVELARIKN AGGKVT                            226
PP2C-Human   HFFTQDHKPS NPLEKERIQN AGGSVM                            183
PP2C-Rabbit  HFFTQDHKPS NPLEKERIQN AGGSVM                            183
PP2C-Rat     HFFTQDHKPS NPLEKERIQN AGGSVM                            183
```

Figure 2

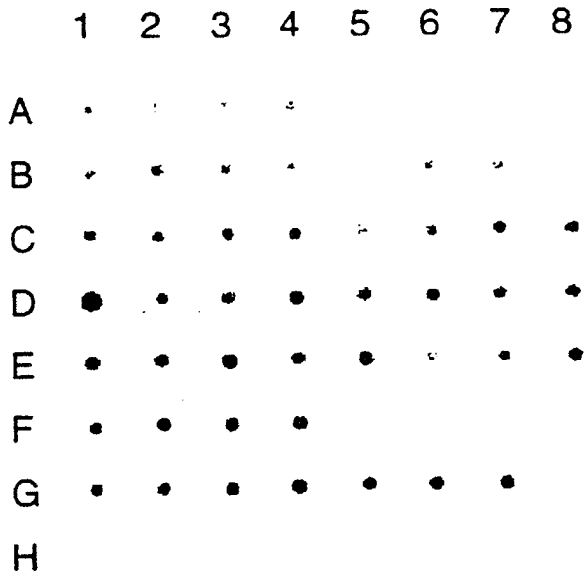
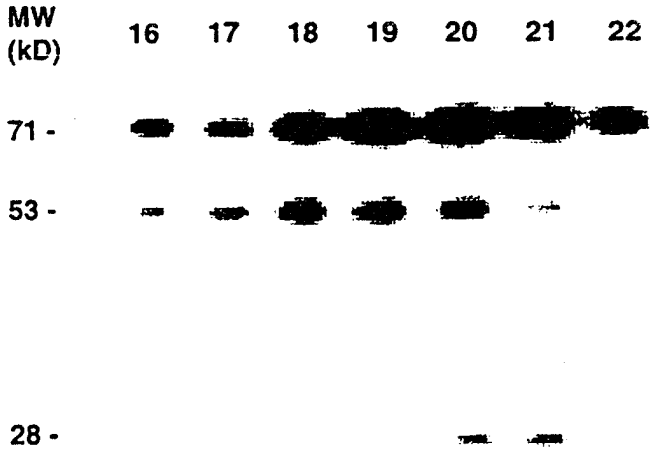


Figure 3



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European Patent Office
Office européen des brevets

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(12) EUROPEAN PATENT APPLICATION

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Designated Extension States:
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(71) Applicant:
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69115 Heidelberg (DE)

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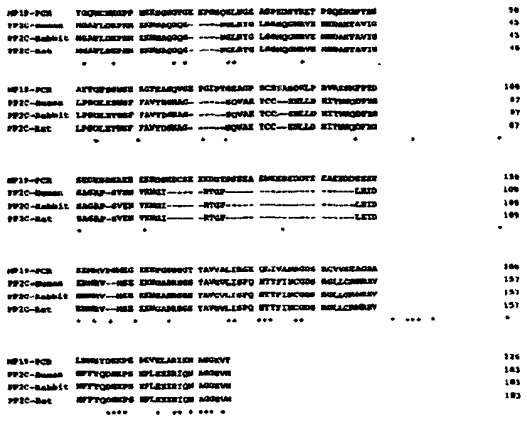
(54) Nucleic acid encoding a human protein phosphatase

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Fig. 1



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which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 10 7346

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	EMBL DATABASE ENTRY MM42383, ACCESSION NUMBER U42383,8 June 1996, XP002085147 * abstract * -& GUTHRIDGE M.A. ET AL: "Induction of expression of growth related genes by FGF-4 in mouse fibroblasts" ONCOGENE, vol. 12, no. 6, 21 March 1996, pages 1267-1278, XP002035691 ---	1-8	C12N15/55 C12N9/16 A61K38/46 C07K16/40 G01N33/577
X	"Database EMBL, Entry BTU81159, Accession number U81159 29.December.1996" EMBL NUCLEOTIDE SEQUENCE, XP002035695 * abstract * -& Y. WANG ET AL: "A Mg2+-dependent, Ca2+- inhibitable Serine/threonine protein phosphatase from bovine brain" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 270, no. 43, 27 October 1995, pages 25607-25612, XP002085148 MD US --- -/--	1-8	

TECHNICAL FIELDS SEARCHED (Int.Cl.6): C12N

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely: 1-9

Claims searched incompletely: 12-17

Claims not searched: 10-11

Reason for the limitation of the search:

Remark: Claims 10-11 completely and 12-17 partially are not searchable because neither an antagonist nor an agonist of the human serine/threonine phosphatase have been described.

Place of search	Date of completion of the search	Examiner
THE HAGUE	20 November 1998	LE CORNEC N.D.R.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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Application Number
EP 98 10 7346

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	WO 97 10796 A (RAMOT-UNIVERSITY AUTHORITY) 27 March 1997 * claims; examples 2,3 * ---	1-9, 12-17	
D,A	D.J. MANN ET AL: "Mammalian protein serine/threonine phosphatase "C: cDNA cloning and comparative analysis of amino acid sequences" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1130, 1992, pages 100-104, XP002085180 * the whole document * ---	1-9	
P,X	S.M. TRAVIS ET AL: "PP2Cgamma: a human protein phosphatase with a unique acidic domain" FEBS LETTERS., vol. 412, no. 3, 4 August 1997, pages 415-419, XP002085149 AMSTERDAM NL * the whole document * -& S.M. TRAVIS: EMBL DABASE ENTRY HSY13936, ACCESSION NUMBER Y13936, 4 August 1997, XP002085150 ---	1-9	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
P,X	WO 97 35018 A (NEW YORK UNIVERSITY) 25 September 1997 * see the whole document especially example 4 page 81 and seqence id no 1 * ---	1-9, 12-17	
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